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BOTANICAL LABORATORY GUIDE

VOL. I

P. H. MELL

Compliments of
the Author.

W

LABORATORY GUIDE

TO

Histological Work

IN

PHÆNOGAMIC BOTANY.

VOLUME I.

BY

P. H. Mell, Ph. D.

PROFESSOR OF GEOLOGY AND BOTANY, ALABAMA POLYTECHNIC INSTITUTE

AUBURN, ALABAMA.

1895.

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PREFACE.

This book was especially written for the use of the botanical students of the Alabama Polytechnic Institute. In its preparation, I have been guided entirely by what seemed to be the average advancement of the students, who report from year to year, for admission to the Botanical Laboratory. The topics treated have not been elaborated, but simply outlines given, so that the student may learn to extend and enlarge his work by references to other sources.

I make no claim of originality, but have only attempted to so arrange the topics, in relation to each other, as to bring them before the minds of my students in the way best suited to the methods in vogue in the Laboratory.

In the preparation of the book, I have made liberal use of the standard books relating to the microscope and to histology; and the leading scientific journals and periodicals have been freely culled from for many valuable items. A list of these works will be found at the end of this Guide and references to them will be noted in the body of the work.

The following firms have placed me under obligations for the permission to use most of the electro-plates illustrating this book.

Bausch & Lomb Optical Company, Rochester, N. Y.; Eastman Kodak Company, Rochester, N. Y.; J. B. Colt & Company, New York; James W. Queen & Company, Philadelphia, Pa.; Buffalo Dental Manufacturing Company; Carl Zeiss, Jena, Germany.

P. H. MELL.

AUBURN, ALA., *June, 1895.*

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LABORATORY GUIDE.

CHAPTER I.

The Room and its Furniture.

The laboratory should be well lighted with large windows, so situated as to supply an ample amount of illumination at all hours of the day. The ventilation must be good; and the heating facilities must be adjusted so that the temperature may be kept as near normal conditions as possible througout the year.

Unless there is a building especially constructed for the laboratory, where suitable rooms are provided for the various departments of work required in botanical research and investigation, it will be necessary to select rooms, at least in this latitude, with southern and western exposures for the reason that direct sunlight is desirable for some of the operations in the laboratory, such, for instance, as photo-micrographic work. During a portion of the day, it is true, the direct sun's rays will penetrate the laboratory to such a degree as to become somewhat unpleasant for certain kinds of work with the microscope, but this difficulty can be largely obviated by placing in the windows white shades through which the light will sift and diffuse through the room. These shades, moreover, will be of great advantage under the management of the skillful microscopist,

since they can be made to distribute the light very much like that reflected from a cloud; and this light will be found well adapted for illuminating purposes while examining the object through the microscope.

The laboratory should be kept as free as possible from all dust which is so deleterious to all microscopic work, as well as to the mounting material.

Illuminating and heating gas must be supplied to each table; and water with properly constructed sinks should be easy of access to each student.

Electricity from the city electric system, or from the best storage battery, is a necessary addition to a well proportioned laboratory. In another part of this work will be found a detailed description concerning the electric outfit.

Laboratory Tables. Years of experience in the teaching of histological botany has convinced the author that the best table adapted to all work required in preparing the object, staining, making the slide and examining the work under the microscope, should be strong and well built; covered with a smooth slate top, and arranged to accommodate not more than two students. The illustration gives a clear idea of such a table. The lower compartment is so divided as to hold two compound and two dissecting microscopes. The drawers are for the dissecting instruments used by the two students. The reagent bottles are placed at the rear of the table on the shelf. Around this shelf, as shown in the cut, is a guard to protect the bottles from persons moving about the room.

The dimensions given in the figure have been found by experience efficient. The height will permit the student to stand while engaged in certain kinds of work, and the elevation is not so great but that ordinary desk stools can be used.

The tables are made narrow so that the least difficulty will be experienced in reaching for the reagents at the rear.

As a matter of convenience for the Professor the drawer-pulls should contain the student's name on a card cover-

ed with glass. There are several patterns of pulls in the market for this purpose.

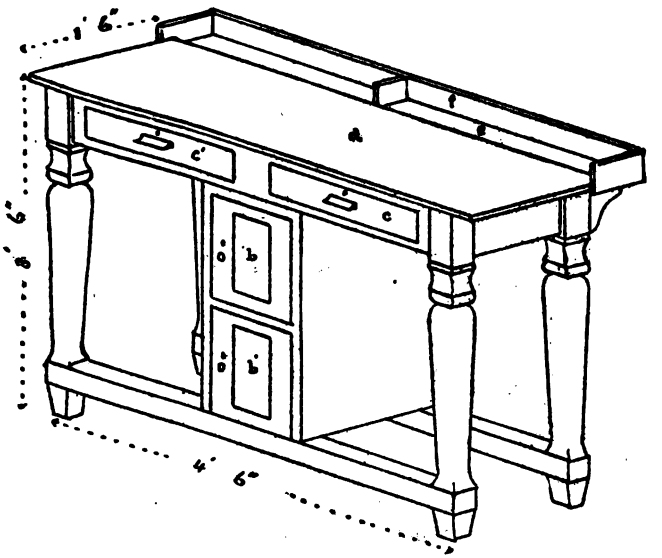


Figure 1. Laboratory Table.

Ample shelvimg covered with glass doors will be found necessary to hold the extra supply of chemicals and apparatus needed to replenish the consumption at the different tables.

Photo-micrography. An adjoining room opening into the main laboratory, should be fitted up for experiments in Photo–micrography. This room must contain the following furniture:

1 - A long narrow table to hold the camera and microscope and attachments. The centre of the room is the best position for this table.

2 - A table to contain the enlarging camera and its outfit.

3 - A stand, similar to a camera stand, to hold the lantern and projection apparatus. This stand should be so constructed that the top can be lowered, elevated or inclined to suit the character of the picture and the relative position of the lantern and the screen. The "Continental stand" will be found well adjusted for this purpose.

4 - A screen sufficiently large to hold the picture projected from the lantern. This screen may be constructed on rollers to permit of being rolled up as a protection against dust, and to remove it out of the way of other opertions.

5 - The windows of this room must be provided with opaqne shades, mounted on spring rollers, so the room may be darkened in a few moments when desired These shades should be made of black material and thick enough to exclude all rays of light. The best way to attach them to the window is to fasten the roller fixtures on the sill and the pulley at the top of the window-frame The cords, after leaving the pulleys, are run to some convenient point in the room where they will be out of the way of other appliances and yet so situated as to be conveniently reached. The rollers should be placed in light-tight boxes, and around the window-frame should be fastened a grooved frame in which the edges of the shade will run when it is raised.

6 - For delicate projection direct from the microscope a large ground glass plate mounted in a frame will be found necessary for satisfactory results.

7 - In one corner of this room a dark-room may be cut off in which to perform all photographic work which cannot be done in white light. This dark-room should be provided with a sink and faucet for supplying ample water; shelving to contain such chemicals that are affected by white light; electric lamps covered by ruby globes, and all the apparatus required to develop the sensitive plates.

8 - Storage batteries to supply the electricity for lighting and heating.

CHAPTER II.

The Microscope.

The microscope, an essential instrument in every laboratory, should be well made out of the best material to enable the student to perform satisfactory work and secure accurate results.

There is no economy in purchasing a "cheap" microscope, but great care and experienced judgment should be exercised in providing each student with a first class instrument made by the most reliable manufacturer. This important fact being well understood and appreciated, it then becomes a matter largely controlled by taste and the character of the work which instrument, from the list of less than a dozen responsible opticians, it is advisable to purchase In this country and abroad excellent microscopes are made by such houses as Bausch & Lomb Optical Company, Zentmayer, Zeiss, Leitz and others.

The cost of the microscope will increase in proportion to the delicacy of the work required, the minuteness of the object under investigation and the advancement of the student in histology. So that, after determining upon the pattern of stand, the most important question to be answered is what kind and number of objectives and oculars are requir-

Figure 2. BAUSCH & LOMB's Dissecting Microscope.

ed. As a general rule it is best to begin with two or three objectives, with oculars to match, and add to the list as the needs demand.

Each student should be provided with

1 - A Simple or Dissecting Microscope,

2 - A Compound Microscope.

The dissecting microscope is a convex glass lens mounted in a metal or rubber holder and attached to a convenient stand with rack and pinion adjustment, mirror and stage, so that the object may be placed under the lens, a steady focus secured and the hands left free to manipulate the dissecting instruments with the least degree of inconvenience. The image produced by this instrument is not inverted as it is in the compound microscope, and hence the dissection can be performed with much greater ease.

One of the best instruments of this character to be obtained in the market is shown in figure 2. It is manufactured by the Bausch & Lomb Optical Company. In the description of this microscope the manufacturers say:

"This instrument is designed for use in laboratories. For this purpose it is made of great stability, with large and delicate bearings in its adjustments. It is entirely of brass highly polished. The base is round and large, and the stage is of the same large dimensions. The upper surface of the stage is black and provided with spring-clips. It has a large central opening holding a ruled glass disk. On its lower side it is grooved to receive hand-rests, if it is desired to attach these. The adjustment is by spiral rack and pinion acting on a large triangular rod. The mirror frame is provided on one side with concave mirror and on the other with a white disk. The mirror is detachable from the base and may be attached to the stage to illuminate opaque objects. The arm is adjustable in the triangular rod and is provided with Society screw. Mounted lenses of 2 inch and 1 inch focus accompany the instrument and may be used singly or in conjunction."

For a long time the single glass employed in the earlier history of this instrument produced spherical aberration and aberration of refrangibility to such a great degree it was found necessary to replace the single lens with what Wollaston, the inventor, termed *doublets*. The two lenses, or doublets, in Wollaston's instrument are so adjusted in relation to each other that they act together as a single glass. They are plano-convex and are separated by means of a diaphragm. Besides overcoming, to a large extent, spherical aberration, by the use of two glasses with a diaphragm interposed, the doublets also increase the distance between the lens and the object, thus permitting more freedom in the manipulation of the dissecting instruments.

Achromatic triplets may also be used on the microscope thus giving a large, flat field and a beautiful definition.

Coddington magnifiers, (fig. 3), which are so conveni-

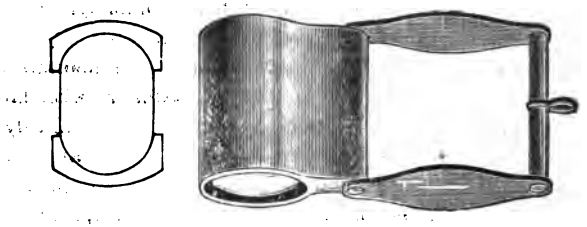


Figure 3. Coddington Lens.

ent for field work and so highly prized by all practical botanists, are also constructed by Messrs Bauch & Lomb to fit this stand. This lens consists of a sphere of glass with a groove cut around the circumference and filled with a dark cement which acts like a diaphragm.

Another most excellent dissecting microscope is made by the Zeiss Optical Company of Germany and is illustrated in figure 4. The manufacturers in describing this instrument say:

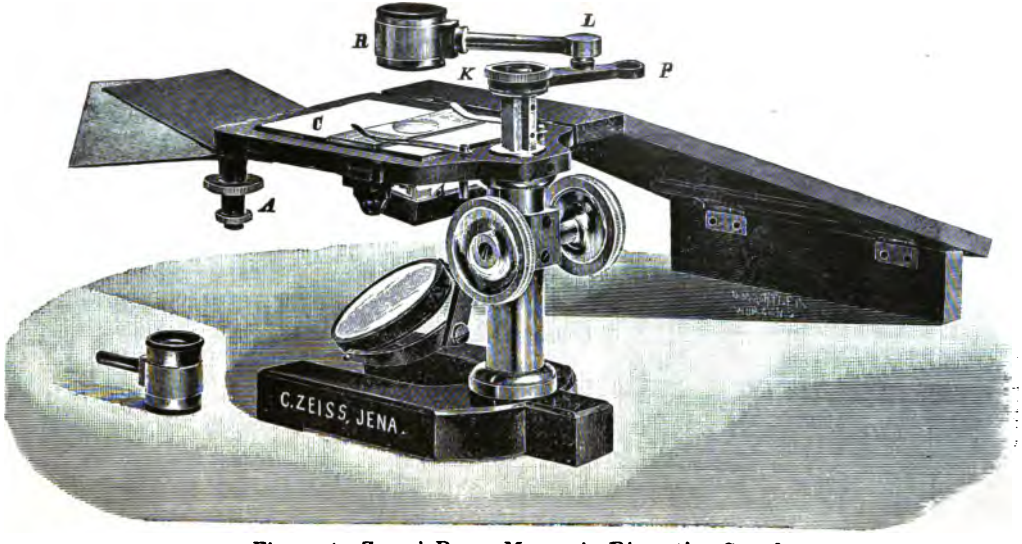


Figure 4. ZEISS' PAUL MAYER'S Dissecting Stand I.

"The stage consists of a large metal frame (10 × 10 cm = 4 × 4 in.) to which is attached wooden folding hand-rests; adjustment by rack and pinion, plane and concave mirrors with universal motions, to which a piece of white paper may be attached by means of a ring so as to produce with low magnifications diffuse illumination.

"The triple dissecting system" which consists "of three achromatic lenses (objectives) and an achromatic concave eye-piece, forms a suitable combination for dissecting and 'teasing' small objects on a slip or in a watch-glass; it may be fixed in the ordinary lens-holder *P*, and a black metal plate with stage opening of 14 mm diameter, which can be closed below by a black or white disc, may be placed under the object into a recess provided in the stage-frame.

"For examining large objects, particularly living aquatic animals, the aplanatic lenses will be found useful." These glasses are ' composed of three cemented lenses, giving relatively long focal distances with large flat field. They fit into a separate arm *LR* which may be inserted into the ordinary lens support at *L* and, by this arrangement, the whole of the stage can be scanned.

"In this case the metal stage is replaced by a glass plate, and the interchangeable white and black discs serve as convenient means of forming a white and black ground.

"A brass plate made to fit the frame serves as a support for small dissecting dishes, which may be cemented to it with paraffin"

Spherical aberration. A few words in reference to this subject may not be out of place in a book of this character in order that the student may form some general idea concerning the principles upon which the Wollaston lens is constructed But, for a full and detailed account of refraction of light and correction of spherical aberration, he is referred to works on optics.

Spherical aberration, so common in single lenses, is due to that property a glass medium has of refracting the rays of light to a greater degree near the circumference than

towards the centre of the glass. For this reason many foci are formed and the light is decomposed into the elementary colors. The violet ray suffers the greatest refraction (its index of refraction being 1.5466); and the red ray, at the other limit of the spectrum, submits to the least refraction (its index being 1.5256). These two rays, therefore, after separating through the medium of the glass, meet the principal axis of the lens at different points, the violet ray nearest the glass at V, (fig. 5), and the red ray at R farthest removed from the lens. The distance between

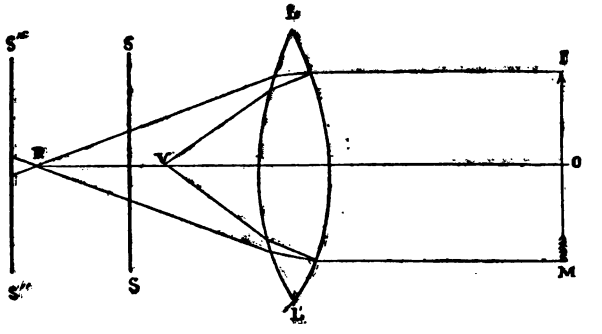


Figure 5. Refraction of Light

these is called *chromatic aberration*. A screen placed at SS will have projected on it a red ring at those points where the red rays strike; and if the screen is moved to a position between V and the lens two colored rings will be formed, the outer one will be red and the inner one violet.

When an object is viewed through a lens producing such a result all colors of the rainbow are discernable and the image thrown on the screen SS becomes very much confused and loses the sharpness of detail so necessary for satisfactory work and accurate conclusions.

Crown and flint glass when combined have the power

to so react on each other that when a ray of light passes through them it is not only refracted and brought to one focus but the decomposition produced by the one glass is overcome by the other and the colors are again recombined into white light. Thus distinctness in the image, and purity of white light are preserved.

The usual form adopted for these lenses is, for crown glass *bi-convex*, and for flint glass *plano-concave*. They are cemented together with Canada balsam. By this combination spherical aberration is largely overcome.

Professor Abbe and Doctor Schott of Jena, Germany, have succeeded in discovering, by means of elaborate and expensive experiments, compounds closely resembling glass, whose properties are so remarkable that a lens for the microscope manufactured from the compound gives an image presenting marvellous distinctness to the outer limits of the field These glasses do not possess silica in their composition, but the silcon is replaced by boron, in the flint series, and by phosphorus in the crown series.

"Professor Abbe has, moreover, introduced several natural substances in the construction of objectives which defy all artificial imitation. Such, for example, is the fluoride of calcium or fluorite of the mineralogist. The index of refraction of this substance is only 1.434, that is to say, considerably less than that of any kind of glass hitherto manufactured, and its dispersion is only half as great.

'If, therefore, a convex lens be made of crown glass, we assume that it is possible to calculate for a concave lens of flint a focal distance such that, when it is applied to the convex lens, recomposes the white light dispersed by the latter without altering its converging properties. A lens thus corrected is calleb *achromatic*."[1]

The scope of this work will not permit of a more extened discussion of refraction. The student, however, is referred to the writings of Professor Abbe, Doctor Van Heurck

1 Dr. Van Heurck's "The Microscope", trans. by W. E. Baxter, page 17

the Journals of the Royal Microscopical Society, as well as other authorities, if he desires detailed information on this important question. The author of this Guide has endeavored simply to give the general principles governing tee work of the microscope in as concise terms as possible with out sacrificing clearness, so that the construction of the instrument may be understood by the student who is just beginning its use.

The Compound Microscope- Figure 6 is a longitudinal section of the instrument and shows that it cnosists of two tubes, one sliding in the other, a stage and stand besides the lenses, called *oculars* and *objectives*. The inner tube carries in the upper end the ocular EY, and to the lower end of the outside tube is screwed the objective, BO. The tubes are blackened on the inside so that all reflection of the light rays after passing through the objective, will be obviated. The sliding of the tubes, one in the other, permits of limited elongation so that the length may be adapted to the character of the objective in use. For instance, the objective made by a German opticiar will not produce satisfactory results when used on an American stand, unless especially adjusted for it. The inner or draw-tube is usually graduated into inches or millimeters to enable the microscopist to secure a known length at will. At she lower end of the draw-tube is a screw thread to hold the auxiliary objective when the *apertometer* is in use determining the numerical and angular aperture of the objectives. The objectives made by Zeiss require a length of tube of 160 mm = 6½ inches, and this length is "reckoned from the contact surface of the objective thread to the upper end of the body on which the eye-piece rests." ZEISS

The following table is given to show how much this matter of tube-length varies with different manufacturers throughout the world:

Reichert of Vienna	160 to 180 mm
Swift & Son of London	165 to 228½ ,,

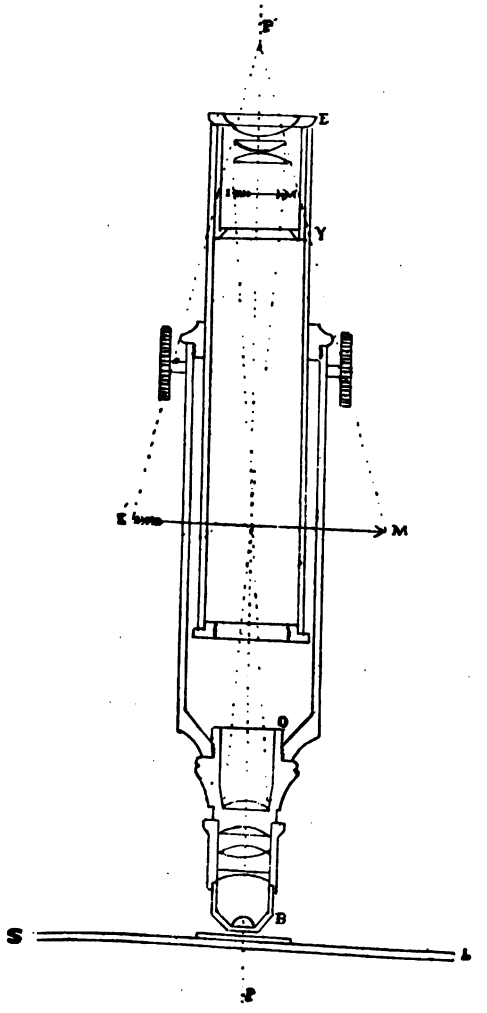


Figure 6. Longitudinal Section of the Microscope.

Zeiss of Jena, Germany	160	mm
Winkel of Gottingen	220	,,
Ross of London	254	,,
Leitz of Wetzlar, Germany	125 to 180	,,
R. & J. Beck of London	254	,,
Grunnow of New York	203	,,
Bausch & Lomb of Rochester, N. Y.	216	,,
Spencer & Co. of Geneva, N. Y	254	,,

The thickness of the cover-glass, at *B* (fig. 6), has a great influence also over the results secured with the objective, because the light is changed in its character and direction after passing through the cover-glass and many rays will be lost unless ths proper adjustment is made in the objective It is best, therefore, to use a cover-glass gauge, when one of the higher series of objectives is on the microscope, and mount with cover-glasses of an estimated thickness.

Another way of determining the thickness of the glass is thus recommended by Zeiss: "The divisions on the milled head of the micrometer screw of stands I–IV furnish a means for exactly registering the vertical movements of the tube. In our present stands each division corresponds to an elevation or depression of the tube in the direction of the optic axis of 0.01 mm. By this means measurements of thickness may be made with a considerable degree of accuracy. The upper and lower surfaces of the object are successively focused and the amount read off on the milled head by the fixed index. In doing this care must be taken to make both adjustments by a rotation of the screw in the same direction. The thickness of an object in air is then equal to the difference between the two readings."

Besides the lengthening of the tube, above indicated, the instrument is also provided with an adjustment which permits the raising and lowering of the entire tube-system and thus bringing the object into focus. This movement is under the control of two mechanisms: one called the *coarse adjustment* and the other the *fine adjustment*. The first is

made by means of rack and pinion movement, noticed in the frontispiece and in figures 6, 7 and 8 nearest the ocular. The second is accomplished by turning the milled head micrometer screw which is generally graduated.

In the cheaper forms of instruments the rack and pinion are wanting and the coarse adjustment is made by sliding the tube system up and down by a twisting motion of the thumb and finger.

The Stand- The base of the stand should be made of strong material, and should maintain a firm and steady position even when the tube of the instrument is inclind at any angle. The Continental and some of the American stands also, have bases in the form of horse shoes, while many of the other makes consist of a three-pronged base.

The Stage- occupies a position on the microscope just underneath the tube (figs. 7 and 8 and the frontispiece) and is firmly fixed to the stand. In its most simple form it consists of a metal table, (see fig. 8) sometimes rectangular and at other times round, with a hole through the centre for the passage of the light from the illuminating system beneath. On the stage are two spring-clips to be used in holding the slide well clamped to the stage. Diaphragms are fitted to the opening in the stage to control the quantity of light.

The Mechanical Stage- placed on top of the simple stage contains the mechanism necessary for producing the required movements to bring all portions of the object readily into view. "The available lateral movement measures 50 mm (= 2 inches) and the stage moves through 35 mm (= 1½ inches) in the direction from front to back. The amounts are read by vernier and scales. The mechanical stage [see frontispiece] is so solidly constructed that possessors of a stand fitted with it may well dispense with the plain vulcanite stage. The mechanism (*LK*) which serves for the lateral movement of the object lifts off after unscrewing the fixing screw *L*, whereby the solid lower part of the stage becomes free. By means of the milled

head *W* this lower part of the stage can be made to move in a forward and backward or—if the whole stage be rotated—in any other desired direction."[1]

The Illuminating Apparatus- This is an important part of the microscope and should be well and accurately made. It is situated beneath the stage. The simplest form is a circular mirror with two reflecting surfaces, and so mounted as to allow of the movement necessary to catch the light from any direction and reflect it through the stage upon the object. One surface of the mirror is concave and the other is plane. The diameter ought to be at least 2 inches so that an ample amount of light can be reflected by it.

Sub-stage Accessories- These, on the most elaborate forms, consist of cylinder and dome diaphragms with Abbe's condensers for passing and condensing the rays of light upon the object. The Abbe system of illumination is quite complicated and is used only on the more expensive instruments. The illustration (fig. 8) gives a clear conception of this apparatus. The principles governing this instrument consists in the action of the short focus condensers, which collect the rays of light from the reflector beneath and concentrates them into a cone of light of large aperture. The condenser is so mounted that the system may be made to approach or recede from the stage, and in this way the degree of illumination is kept under perfect control. Between the condenser and the reflector is an iris diaphragm which is very essential to the system in reducing or enlarging the cone of light. This diaphragm is controlled by mesns of a pin *d*, fig. 9. In this illustration, *a* is the condenser system of 1.20 numerical aperture; *b* is the condenser system of 1.40 numerical aperture; *c* is the cylinder diaphragm; and *e* is the milled head for throwing the diaphragm out of centre. The "swing-out condenser" is shown more in detail in figure 10 It is thus described

1 Carl Zeiss' "Microscopes and Microscopical Accessories," page 38.

Figure 7. Zeiss' Small Stand VIa

Figure 7. Zeiss' Small Stand VIa

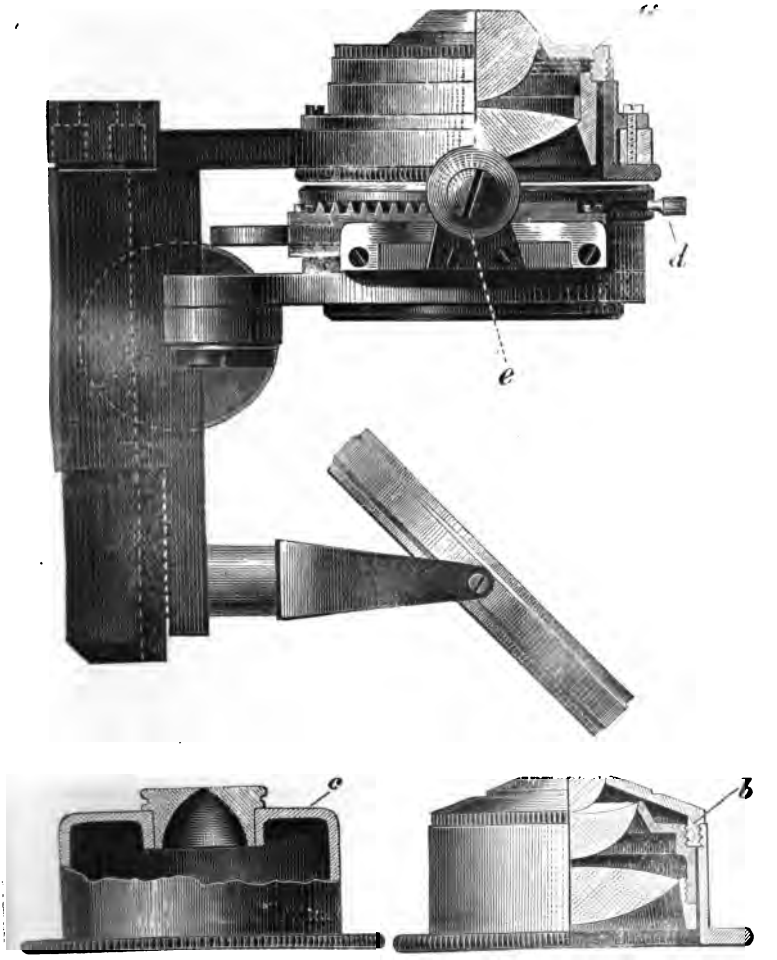


Figure 9.¹ Abbe's Illuminating Apparatus

by the manufacturer: "The condenser-system is by a suitable mechanism so connected to the sleeve enveloping it that, after swinging the diaphragm carrier *D* aside (towards the right of the observer), it may by means of the lever *H* projecting from under the stage be swung downwards about the axis *Q* and to the left about the pivot *Z*. When the condenser is not being used the width of the illuminating cone is regulated by means of the *Iris Cylinder Diaphragm* which is permanently attached to the apparatus and is actuated by the small pin *K* seen on the right. The iris diaphragm is so shaped that the edge of its smallest opening closely approaches the object slide."[1]

The iris diaphragm in the sub-stage is the best contrivance for controlling the light of any diaphragm now known. There are many advantages in its use, among which may be mentioned, the time and inconvenience saved in substituting one metal disc for another, as is required in the old system; the gradual exclusion of the rays as the iris is contracted, thus producing an effect in the appearance of the object.

Figure 10. Zeiss' Swing-out Condenser.

1 Carl Zeiss' "Microscopes and Microscopical Accessories," page 61.

The Revolving Nose-piece- This is a mechanical devise for enabling the manipulator to rapidly and conveniently change from one objective to another. The nose piece (fig 12) is screwed to the lower end of the tube

Figure 12. Nose-piece.

and is so constructed as to hold two, three or four objectives at the same time. By revolving the instrument each objective is immediately brought into use, requiring but little adjustment of the microscope to bring out the image clear and distinct.

CHAPTER III.

THE MICROSCOPE, *Continued.*

Sliding Objective-changers.—Dr. Roderick Zeiss, of Germany, has devised an apparatus (figs. 13 and 14) which is considered, by most microscopists, to be superior to the revolving nose-piece. This instrument is called the "*sliding objective-changer.*" The appliance consists of two parts: (*1*) *the tube-slide;* (*2*) *the objective-slide.* The first is permanently screwed to the lower end of the tube, in the same manner explained with the nose-piece. The lower part of the tube-slide contains a grooved plane inclined to the optic axis of the microscope. The objective slide contains a similar grooved plane inclined also to the optic axis, and when the objective is pushed into working position the inclined planes permit the lens to approach gradually towards the slide containing the object without danger of breaking the glasses. This sliding objective-changer is adapted and corrected to fit certain objectives and microscopes, but it may be altered to suit any standard microscope and lens by simply adjusting the screws which move at right angles to each other so that centering may be accomplished in either direction.

Figure 13.

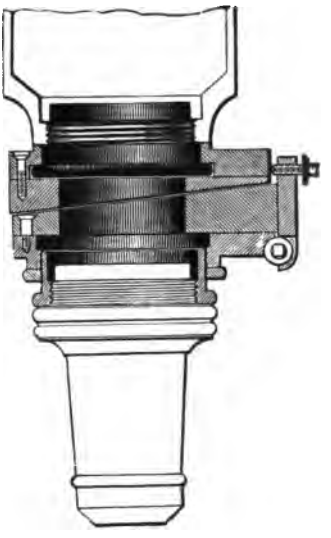


Figure 14.

Zeiss' Sliding Objective Changers

The Oculars- There are two kinds of oculars, or eye-pieces, which are termed *positive* and *negative*. The first comprises those glasses in which the real inverted image is made ontside the ocular, while the latter have the image produced within the glass. The positive eye-piece is a simple magnifier and may be used in place of the ordinary hand-glass; the negative lens cannot be so used.

The most important oculars are:

1 - Huyghenian oculars (fig. 15). These consist of two plano-convex lenses, one at each end of the brass tube. The upper lens has its plane surface towards the observer,

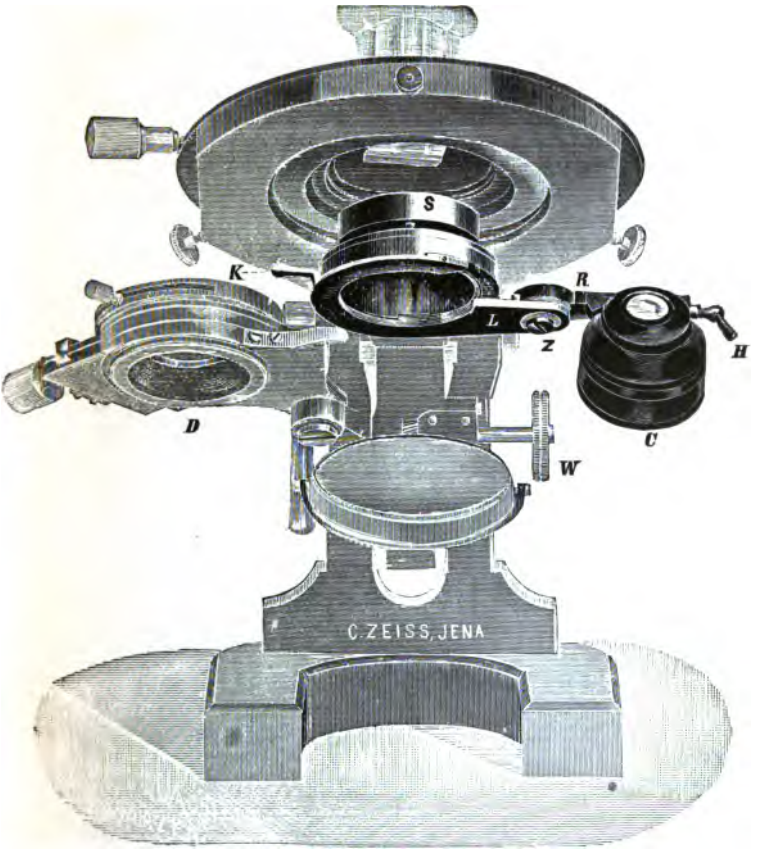


Figure 11. Zeiss' Swing-out Condenser Attached to the Microscope.

and the lower lens has its convex surface towards the object. The upper is called the *eye-glass* and the lower is known as the *field* or *collecting-glass*. Between these lenses is placed a diaphragm near the focus of the eye-glass This ocular belongs to the negative type and is used in most microscopes made on the American and Continental plans. There are two kinds of eye-pieces, viz:

Figure 15.
Huyghenian Ocular.

a - The Huyghenian proper, which is generally used on American microscopes (fig. 15).

b - The Continental form which is used on all German and French instruments (fig.17).

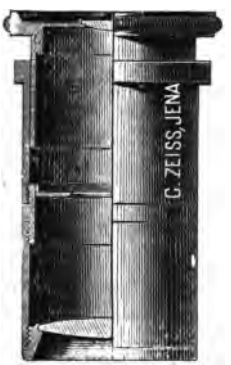


Figure 16.
Micrometer Eye-piece.

Figure 17.
Continental Ocular.

2 - Ramsden's positive ocular. These eye-pieces do not distort the image as much as is the case with the Huyghenian oculars, and they are, therefore, more suitable for mi-

crometer measurements. They consist of two plano-convex lenses, with the convex surface turned towards each other.

3 - Compensating oculars. The eye-pieces by this name are manufactured by Zeiss, and are adjusted for use with the apochromatic objectives. Two classes are made:

a - Searching oculars which are used for reducing to the lowest possible degree the magnification produced by the objective and facilitating the location of the image.

b - Working eye-pieces. These are also the results of the inventive genius of Zeiss, and were designed especially to be used in conjunction with the apochromatic objectives made by the same house. They are numbered 1, 2, 4, 6, 8, 12 and 18. "The number which denotes how many times an eye-piece, when used at a given tube-length, increases the initial magnifying power of the objective affords the measure of the eye-piece magnification and, at the same time, furnishes the figures for their rational numeration. On this basis the series of our compensating eye-pieces is arranged according to their magnifying powers. The magnification obtained by combining a compensating eye-piece with any apochromatic objective is found by multiplying its number by the initial magnification of the objective. An objective of 3.0 mm focus. for example, yields in itself a magnfication of 83 3 (calculated for the conventional distance of vision of 250 mm); eyepiece 12 therefore gives with this objective a magnification of 12 x 83.3 = 1000 diameters." — Zeiss.

The compensated oculars are corrected for aberration outside the axis, and the upper lens is plano-convex and simple, while the bi-concave lens beneath it is complex.

4 Projection oculars. These eye-pieces are used for projecting an image on a screen or for photographic purposes in making photo-micrographs. They are corrected so that they may be used with the apochromatic objectives, and the magnification is determined in the same manner as used in the case of the compensating oculars. The magnifi-

cation of the image on the screen, or ground glass of the camera, may be determined by dividing the distance between the ocular and the image by the focal length of the objective and multiplying the result by the number stamped on the ocular. The distance must be expressed in millimeters, because the other factors of the equation are so expressed. This will hold good for long distances, but for short spaces the results are not so accurate.

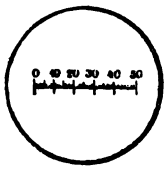
The field is limited by the diaphragm between the two systems of lenses. The projection lens is so mounted that it may be moved from or towards the diaphragm. Before beginning photographic operations, it is best to focus on the screen, or ground-glass, by extending or pushing into the tube the projection-glass until the outlines of the diaphragm are distinctly marked, and then the apparatus is adjusted for work. If the microscope is near the screen it will be necessary to extend the projection-glass from the diaphragm.

In the use of the projection ocular care must be exercised in adapting it to the objectives. The oculars made by Zeiss are numbered 2, 4, 3 and 6. The 2 and 4 systems are adjusted for the Continental tubes, or 160 mm, and they have an amplification of 2 and 4 times. The 3 and 6 oculars are to be used with the English tube of 250 mm (10 inches). They are specially designed for the apochromatic objectives and should only be used with them when results of the highest order are required. In their use also care should be exercised that the length of the tube is fixed at that figure for which the oculars are manufactured.

The images produced by these eye-pieces are remarkably clear and accurate, and the photographs made by them are exact reproductions of the images seen by the observer through the ordinary ocular.

5 – Micrometer ocular. This is an eye-piece made according to the Huyghennian pattern with the eye-lens adjustable so that a focus may be secured on the measurements of the micrometer resting on the diaphragm between

the eye-lens and the field-glass. Figure 16 gives a correct idea of this ocular. The micrometer that accompanies this instrument is shown in figure 18, which consists of a disc of glass divided into arbitrary lines which, when compared with the stage micrometer, will give the size of the image produced by the object. It is best to construct a table for each objective, with the assistance of the stage micrometer, so that the values of the divisions on the eye-piece micrometer may be quickly determined.



Eye-piece micrometer.

Figure 18.

Camera-Lucida, after Abbe. This is an appliance attached to the ocular of the microscope to be used in drawing the image of the object which is under investigation. It consists of a cube of glass cut into two prisms, one of which is silvered In the centre of the silvered surface is left a small opening after which the two prisms are cemented together. This compound prism is placed over the ocular and carefully adjusted. The light from the object is caught by the prism and turned in a horizontal direction, through smoked glass, to the surface of a mirror from which it is reflected to a piece of paper at the base of the microscope where the image is projected and drawn. The use of this apparatus will be fully described in the chapter devoted to drawing and photography.

There are other camera-lucidas to be found on the market which are made on the same general principles, but Abbe's pattern is so well adapted for the purpose, and in some particulars is so superior to all others, we will not delay to describe the other styles but wi l refer the student to larger works on miesoscopic apparatus.

The Objective- This is the most important part of the microscope; the material of which it is made must be of the best, and its manufacture must be by the most skillful opticians. Great skill has been developed in America and abroad in calculating the lenses for objectives, and workmen of remarkable expertness have been trained in their manufacture.

In the selection, therefore, of a microscope the student with limited means should purchase a good stand with few, if any, accessories on the stage, and put most of his money in one or two of the best objectives, of comparative low power, to be found in the market. As his work advances in interest, and his income will permit, he may add gradually to his list of objectives, so that in the course of time he will possess an outfit with which he will be able to execute first class work of a most delicate character.

Objectives for compound microscopes are all achromatic. That is to say, they are so constructed that the colored rays decomposed out of white light by the first lens are recomposed by the next lens into white light and the image is produced clear and sharp. When the *three* principal rays of the spectrum are united by the lens system of the objective in one point of the axis and the correction of *two* different colors has been at the same time accomplished, such an objective is called *Apochromatic.* These are now manufactured by Zeiss of Germany and they are the finest, most expensive and most satisfactory objectives to be obtained in the world. The image made by them is distinct and sharp to the outer limits of the field, and the results seem to be most perfect in all respects.

Objectives are generally divided into two classes:

1 - Dry objectives, and

2 - Immersion objectives.

The first, or dry series, are used for examining objects in air, that is to say, there is no liquid interposed between the lens and the slide.

The second class requires the interposition of a drop of water or oil between the lens and the slide-cover, to catch those rays of light which are considerably refracted by the cover glass, and, without the aid of the liquid, would pass beyond the reach of the objective.

The immersion objectives constructed for oil are called *homogeneous*. Cedar oil is usually found best adapted for these glasses This oil has the same index of refraction as crown glass, viz: 1.515. The index of refraction of water (1.336) being lower than oil some of the rays are not caught by it, and hence it is not so useful as an immersion medium. The homogeneous objectives have a greater frontal distance and give clearer images than are possible with dry objectives.

In the manufacture of objectives by different houses in this country and abroad the shape, size and screw-threads of the lens mounts were almost as numerous in variety as was the number of manufacturers. But as the demand for delicate and multiplied work with the microscope increased in different parts of the world came also the demand, on the part of the microscopists, for greater facilities in handling the instrument. It was then that the manufacturers realized the importance of making certain parts of the instrument, made at one factory, interchangeable with similar portions made by another firm.

The most important part of the microscope subjected to this evolution was the screw-thread used in attaching the objective to the tube of the instrument. Even in this late day we have the *German thread; the English thread* and the *American thread.* Some years since, however, the Royal Microscopical Society of England devised a screw which is now called "*The Society's Screw*", and most of the opticians in America and abroad have adopted it, so that now stands purchased in one country may receive objectives made in another portion of the world.

Objectives were generally designated by letters, but

within a few years, it has become customary to mark them with the number indicating the focal distance of the lens. And it would be of great advantage if all objectives would also contain characters to show the *angular aperture*. This is the angle included between the two rays of light emanating from the axil point of the object and entering the objective. "In general, the angle increases with the size of the lenses forming the odjective and the shortness of the equivalent focal distance. If all objectives were dry or all water, or homogeneous immersion, a comparison of an angular aperture would give one a good idea of the relative number of image-forming rays transmitted by different objectives; but as some are dry, others are water and still others are homogeneous immersion, one can see at a glance that, other things being equal, the dry objective receives less light than the water immersion, and the water immersion less than the homogeneous immersion. In order to render comparison accurate between different kinds of objectives, Professor Abbe takes into consideration the rays actually passing from the back combination of the objective to form the real image; he thus takes into account the medium in front of the objective as well as the angular aperture."[1]

Numerical Aperture- (Indicated in works on the microscope by the letters *N. A.*), was introduced by Professor Abbe to designate "the capacity of an optical instrument for receiving rays from the object and transmitting them to the image, and the aperture of a microscope objective is, therefore, determined by the ratio between its focal length and the diameter of the emergent pencil at the point of its emergence, that is the utilized diameter of single-lens objective or of the back lens of a compound objective."[2] The formula in use to determine the values of numerical apertures is stated as follows:

$$N.A. = n \sin. u.$$

1 "The Microscope", by S. H. Gage, page 7.
2 Ibid.

in which n represents the index of refraction of the substance in front of the objective, and u the half angle of the aperture.

It will be of some importance to the student to know before making a purchase, how many objectives are required to enable him to perform his work with satisfaction and accuracy. Experience has demonstrated that the complete outfit for all investigations, from the ordinary to the most delicate work, must consist of the following objectives:

2 Inches, (48 mm).
1 Inch, (24 mm).
1–2 Inch (12 mm).
1–4 Inch (6 mm).
1-10 Inch, (1.5 mm).

In this series 1-6 and 1-12 inch may replace the 1-4 and 1-10 inch. The 1-10 and 1-12 inch objectives should be of the immersion type. The apochromatic lens, as has been stated, is the best, and it will be the part of wisdom to purchase this kind of objective.

For the use of students, who are not far advanced in histology, the following objectives will be found sufficient:

1 Inch (14 mm).
1-5 or 1-6 Inch.

CHAPTER IV.

Apparatus and Accessories.

Besides the microscope mentioned in preceding chapters, the laboratory should also contain the following accessories, a set for each student:

1 - The Microtome.—There are several first class instruments for cutting sections sold by some of the leading manufacturers, and, since they are all made on the same principle, it will be only necessary to describe two or three which possess the most convenient facilities for work.

The microtome is an instrument constructed for cutting thin sections of animal and vegetable tissues, so that a satisfactory examination of the cells may be made with the microscope. The essential features of this instrument are a keen edged knife firmly held on a sliding carrier and a clamp for holding the specimen while the sections are being cut.

1 - Bausch & Lomb's Microtome. This well known firm manufacture two styles of section cutters, one called "The Laboratory Microtome," and the other "The Student's Microtome." The first is larger than the latter, and it also contains a few more details. The cut (fig. 19) represents the Student's Microtome, and it gives a very clear idea of the instrument.

"In both sizes the base, curved arm, upright and V-shaped beds are made of one continuous casting, thus insuring extreme rigidity, without excessive weight. The knife carrying block is fitted in the angular way in such a manner that the knife moves steadily through it without deviating from its plane and without requiring any extra pressure. Stop screws with soft rubber cushions, are fastened at the ends of the way which serve to overcome any sudden concussion and thus prevent a vibration of the knife. The upper surface of the block is provided on its entire length with a grooved slot, to which is fitted a sliding thumb-screw, so that the knife may be fastened at any point upon it.

"The front vertical bed is planed and polished, and is arranged with a grooved slot its full length. The adjustable carriage is fitted to it, and may be securely fastened at any point upon it by means of two heavy screws The tightening pin for these, when not in use, has a receptacle in the solid bed. It will thus be seen that whether it is desired to make a straight or very oblique cut, the carriage with object may be placed at such a point where it is the most convenient in its relation to the cutting edge.

"To the carriage are directly fitted the micrometer screw with graduated disk and a slide which is acted upon by the former. The slide is provided with a grooved slot, which admits of the quick vertical adjustment of the universal joint and clamp for specimens of different lengths.

"A permanent lever which is provided with a reversible pawl, acts in either direction on the milled edge of the disk, and works within a definite limit, which is prescribed by two set screws, attached to the arc of a fixed circle. The divisions read to *mikrom*.[1]

"A drip-pan is countersunk in the upper surface of the bed, and is easily removable for the purpose of cleaning.

1 The usual method for spelling this word is Micron. It represents the unit of measure in Micrometry and is the thousandth of a millimeter (0.001 mm). The character adopted to indicate this measure is the Greek letter mu.

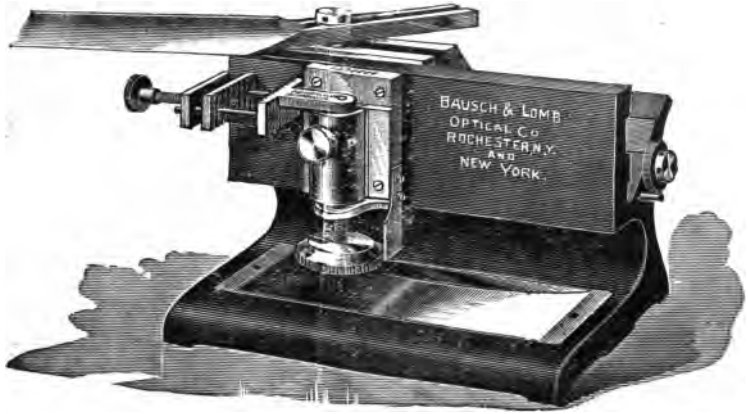


Figure 19. Bausch & Lomb's Student Microtome.
(Cut one-third of actual size.)

"It is impossible to use the knife with a thin edge for all kinds of work, it will invariably nick or be otherwise damaged when such a one is used on hard substances; a knife with an edge not ground as thin as they usually are, should be used expressly for cutting hard substances."[1]

2- The Providence or King's Microtome. This instrument was first devised by a party of microscopists living in and near Providence, Rhode Island, and was afterwards greatly improved by the alteration of important parts in its construction through the ingenuity of Rev. J. D. King of Cottage City, Mass. It consists of a solid iron bed which is clamped to the table by a screw. To the top of this iron base is fastened permanently a brass plate containing in its centre and rising above its surface a cylindrical tube 29 mm in diameter, and projecting above the surface of the brass plate 10 mm. Inside of this well is a piston working in a sleeve in the larger well. The King carrrier is a strong brass plate sliding in grooves in the iron base, and containing in its upper surface a number of small threaded holes in which three screws work; one through the handle of the knife and the other two in a metal bar passing across the opposite end of the knife. The numerous holes in the carriage permit the adjustment of the knife-edge at any desired angle. A long slot is made in the centre of the carriage in which the well or object-holder passes.

This microtome is well adapted for cutting sections of hard substances and there is no irregularity in the cut surface because the knife is so firmly clamped as to prevent the least perceptible deflection.

There are other microtomes possessing excellent merit, and they are well thought of in the best laboratories, and preferred by some leading microscopists to those described above. A few of these instruments will be only mentioned since the general principles are the same for all. The student, however, is referred to the works of Heurck, Behren, Beale, Carpenter, Frey and others, where he will find full descriptions of all the best known machines.

1 Bausch & Lomb's Catalogue, "Columbian edition", page 77.

3 - Beck's New Universal Microtome.
4 - Minot's Automatic Microtome.
5 - Renchert's Microtome.
6 - Zyder's Automatic Microtome.
7 - Taylor's Freezing Microtome.
8 - Thoma's Microtome.

2 - Forceps - These are used for grasping the cover-glasses and holding small objects. The tips should be grooved on the inside so that the object may not slip out. There should be at least two or three instruments on each student's desk – one with straight tips and the other bent.

3 - Scissors - These must be of the very finest quality and may be either straight or curved, although both forms will be found convenient. The usual length is 4½ inches. The edges must be kept keen when fine work is desired.

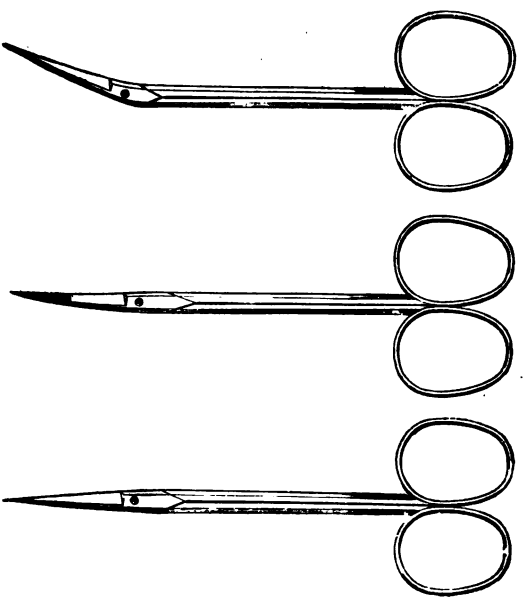


Figure 10, Dissecting Scissors,

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4-Needle-holders-The needles are for dissecting purposes, and the sizes generally used are Nos. 11 and 12. The holders are so constructed that one size of needle may be substituted for another by means of a clamp at the end of the holder. Needles may be purchased from the dealers containing straight, bent and flattened points of various shapes.

5-Scalpels-Dissection knives are essential in microscopic work, and various shapes of blades are now sold by the manufacturers. The one illustrated in the cut is the best form for general use. It has an ebony handle and contains a blade 1¾ inch long. The edge should be as keen as a razor at all times; and the surface of the knife must be dolished after each using so that rust may not spoil its working properties. The knife must be made of the finest English steel, well tempered, so that it will hold its edge under ordinary careful treatment.

Fig. 21
Dissecting Needles

6-Section-lifters-These are made of brass, nickel plated, ¾ of an inch wide on the spatula end, and 5¼ inches in length. This instrument is used for transferring the sections from one vessel to another without damaging their delicate structure.

7-Spring-clips or Compressors- These are made of wire, nickel plated, and are used for holding the cover-glass in position over the object while the slide is being cleaned and the mounting medium is hardening previous to spinning the final finishing ring.

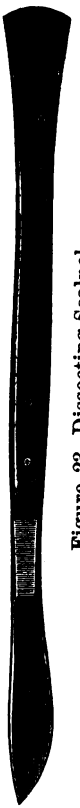


Figure 23. Dissecting Scalpel.

8-Brushes- Camel's hair pencils in quills and mounted on short handles will be found useful in numerous manipulations. In transferring the delicate sections from the microtome knife to the watch-glasses; in moistening the surface of the knife while the sections are being cut; in dusting off cover-glasses and slips; in spinning rings of gum on the slips to seal the cover-glass. The pencils should be in assorted sizes, from Nos. 1 to 8.

9-Bell Glasses- These are convenient for preserving the slides from dust during the process of mounting. These glasses should be from 10 to 30 cm in diameter. A number of bell glasses on the work table will be found very useful.

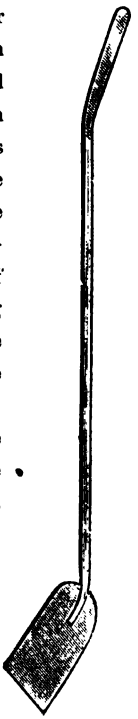


Figure 22 Section Lifter.

10-Glass Benches- While the mounts are undergoing the various processes it becomes necessary to set them aside under certain conditions, and, in a laboratory where many slides are being made, the space on the table must be economized. These benches, therefore, when piled one on top of the other, and covered with a bell glass will hold a greater number

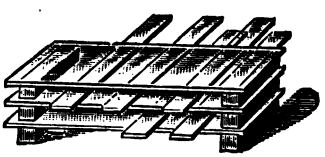


Figure 24. Glass Benches.

of slides than could be possibly stored under the bell-glass without them. The usual length is 130 mm and 55 mm wide. When these benches are used the bell-glass should be procured of sufficient dimensions to accomodate them.

The Petrie dishes will be found admirably adapted for holding these dishes; or a set of crystallization glass vessels with flat bottoms and perpendicular sides, inverted on a glass plate, will also serve a good purpose in this connection.

11 - A Small Wash-bottle- This apparatus, so necessary in a chemical laboratory, will be found useful on the microscopist's table for holding distilled water and dilute alcohol.

12 - Watch-glasses- There are several shapes of watch-glasses sold in the market, but the author has

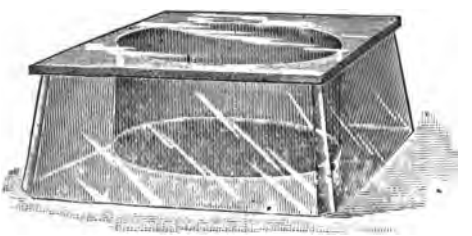


Figure 25. Embryological Watch-glass.

found, after much experience, that the form illustrated in figure 25 is very well adapted for general use in the laboratory. The vessel is covered with a glass plate and the bottom is flat so that the light is but little refracted. In many embryological manipulations, where the watch-glass is used on the stage of the microscope, the light reflected from the mirror below is not enough refrated by the bottom of the watch-glass to materially affect the results of the observations. The rectangular shape also renders the handling of the vessel more convenient than is the case with the circular forms.

Another pattern of watch-glass, called "Syracuse solid watch-glass," is very good and it possesses certain properties of special merit. The edges and bottom are accurately ground so that when one glass is placed on top of another air-tight covers are thus provided for each vessel. This form will, therefore, take up less room on the table. which is quite a desideratum.

These two styles of glasses are made by the Bausch & Lomb Optical Company out of the best quality of material.

13 - Glass Pipettes- These tubes are made with one end drawn to a point and on the other end is a rubber bulb They are useful for transferring a drop of fluid to the places desired in the manipulations. By pressing the bulb and immersing the point in the liquid and then releasing the pressure the fluid will rise in the tube and may be transported with perfect safety. By another slight pressure a drop or more may be excluded when desired.

14 - Erlenmeyer's Flasks- These vessels, in various sizes, will be found serviceable in the laboratory. A supply of beakers will also be required for numerous purposes.

15 - Mortars- Mortars made of iron, wedgewood and glass should be placed in the laboratory for general use in the preparation of staining and mounting media.

16 - Thoma's Dehydrating Apparatus- This, or a similar contrivance, will be found useful in the preparation of the sections while staining, hardening or dehydrating. The Thoma apparatus consists of a museum jar 9×9 inches containing a diaphragm of perforated hard rubber suspended three inches from the top. Through the holes, of various sizes, in this diaphragm. are passed the dehydrating tubes which are six inches long, and in which are placed the sections to be treated. On the lower ends of these tubes are fastened chamois skins. If dehydration is to be accomplished the tubes are filled with 50 per cent. alcohol, and in the jar is placed 95 per cent alcohol. By osmotic action the two will become the same strength. and

the tissues can then be treated with absolute alcohol, if complete dehydration is desired. By this method of treatment the tissues do not shrink since the changes are not sudden. An addition of calcium chloride to the jar's contents absorbs the water and the quality of the alcohol is not depreciated. The rapidity of the dehydrating may be increased or diminished by the degree of thickness of the chamois skin on the tubes.

17 - Turntable. This is one of the absolute essentials to each work-table. There are several patterns sold

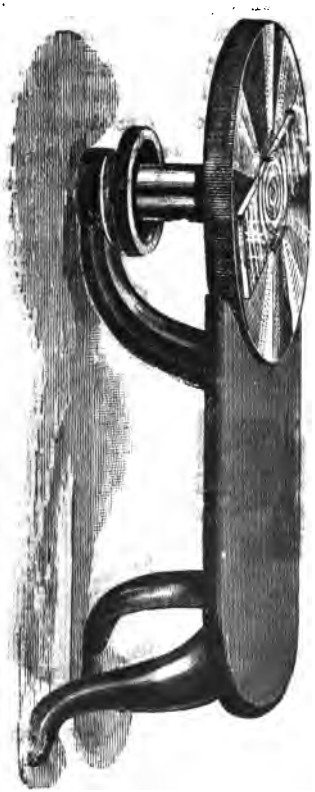


Figure 26. National Centering Turn-table.

by dealers in microscopic apparatus, differing from each other in minor details, but the form illustrated in the cut (fig. 26) contains all the important principles of the turntable. This is the American table, and, for all ordinary purposes it subserves the ends desired. Messrs Bausch & Lomb have devised a table with a movable hand-rest which projects partly over the circle of the table but not in contact with it, thus giving a support for the hand which is very desirable in those manipulations where steadiness of the brush is necessary to produce neat and accurate work Glass slips can be quickly centered on this table by means of adjusting pins, and the usual spring clips are also present to hold the clip firmly to the table.

18-Carbonic acid Freezing Apparatus

Some kinds of tissue must be hardened before they can be properly cut by the knife of the microtome. This may be accomplished by freezing. There are several methods devised for securing this end. Among the number may be

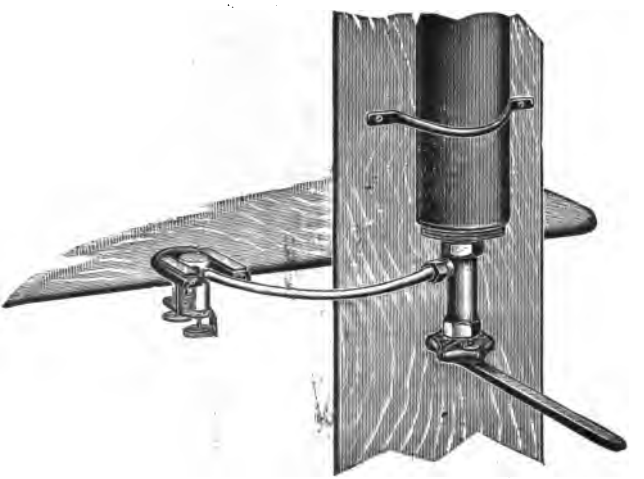


Figure 27 Carbonic Acid Gas Freezer.

mentioned the one illustrated in fig. 27, which gives a very fair idea of the apparatus.

"It commends itself for rapidity of action and economy in the consumption of the freezing liquid. It is composed of the following parts:

"1 - A cylinder (5 9-16 inches outside diameter, 44 inches high) containing compressed carbonic acid gas, provided with hood to protect the valve during transportation,

"2 - A bracket and two iron straps with screws for fastening the cylinder against the wall or other support.

"3 - A bronze cap with nipple of small opening.

"4 - A wrench with attached screws; the opening in this is provided to unscrew bronze cap from the cylinder and attach bronze cap with nipple. The screws are provided to attach the valve wheel,

"5 - A rubber hose; one end of this is attached to the nipple and is fastened thereto by means of wire or twine, the other end is slipped on to the freezer, this latter is to be fastened to a microtome." [1]

Dr. Thomas Taylor, Microscopist to the Department of Agriculture, Washington, D. C., has also devised a freezing microtome which bears his name. It is quite effective for the purposes for which it is intended to be used. For a clear and full description of this apparatus the student is referred to Hervey's translation of Behrens's "Guide to the Microscope in Botany," page 191.

The process of hardening vegetable tissue by freezing is greatly to be preferred to the older methods of cutting imbedded tissue in paraffin, because the work is much quicker done, with less chance of damaging delicate cells and tissue.

19 - Paraffin Imbedding Box. Where imbedding is preferred the apparatus shown in fig. 28 is most convenient. It consists of two triangular pieces of type metal, about 20 mm high, with a glass plate to form the

1 Bausch & Lomb Optical Company, Rochester, N. Y.

base. To use the instrument, wet the glass plate with glycerine and gently warm it; now place the box on the plate, adjusted to the dimensions desired, and pour in the paraf-

Figure 28. Paraffin Imbedding Box.

fin. Before the paraffin is cooled the specimen is immersed in it in the position desired; and after the preparation becomes hard it may then be taken from the metal box and transferred to the microtome to be cut into sections

20 Paraffin Bath. The bath devised by Dr. W. S. Miller of the University of Wisconsin, is among the best of its kind. It consists of a rectangular, polished copper vessel with a sheet iron base, to prevent the burning out of the copper. The top of the bath contains the following apparatus:

1. Two cups, one 2¼ inches in diameter and the same in depth, and the other 2½ inches in diameter, shallow, and shaped somewhat like a watch-glass.

2. Five bottles, to be used in various preparations of the imbedding material. As will be noticed in the cut, the bottles each occupy a receptacle sunk in the bath so that uniform heat may be secured.

3. Two drawers, or trays, in which the slides may be placed to dry and harden the gums.

4 - A shelf upon which the forceps, needles and other instruments used about the bath may be placed and kept at the same temperature of the paraffin.

5 – There is an opening at one end of the bath in which the Bunsen burner is placed.

6 - A thermometer to keep the temperature uniform.

Figure 29. Miller's Improved Paraffin Bath.

Another bath of value, devised by Dr. Reeves, is illustrated in fig. 30. It consists of heavy polished copper, with paraffin cup and stirrer and thermometer. The vessel is 8½ inches high and 10 inches wide.

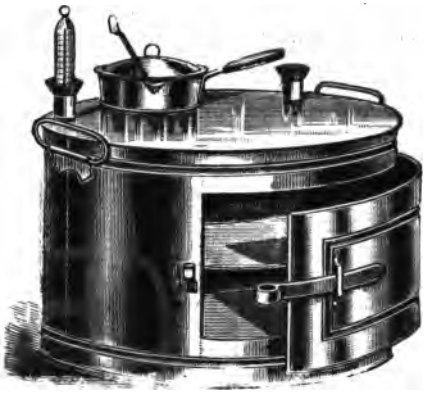


Figure 30. Water Bath and Oven.

In the February number, 1895, page 120, of the Journal of the Royal Microscopical Society is the description of an "apparatus for paraffin imbedding" by Dr. A. Kolossow. This instrument is very well suited for the purpose, and is provided with a number of subsiduary parts which enable the microscopist to accomplish much in the imbedding line not so conveniently performed with some other baths. The description given in the Journal states that "the apparatus consists of a quadrangular box of copper tinned on its inner surface. It is about 24 cm broad and 26 cm high, and it is divded by a horizontal partition into two compartments, the upper being 10 cm, the lower 16 cm high. In the upper one and in the lower are four spaces for the reception of drawers made of nickeled copper. The drawer-cases are supported by upright pieces. On the top of the front of the box is a cup-board, behind which is a free space of 10 cm. To the bottom of the cup-board and to the top of the box a sort of damper, in which is a double row of holes 15 cm in diameter, is fitted. At the top left hand corner are two openings, both of which communicate with the lower department, and through which it can be filled with water.

"In one is fixed a thermometer and in the other a gas-regulator. The level of the water is regulated by a tube. At the bottom of the box is a tap for letting off the water."[1]

Two other thermometers are placed in the apparatus, one, the bulb of which is immersed in a vessel of water in the cup-board, gives the temperature of that compartment, and the other is attached to the top drawer. By this provision three temperatures are procured with differences of about 8° C, which is an important factor in paraffin imbedding.

R. Jung, of Heidelberg, manufactures a bath which is convenient and useful in imbedding manipulations. This instrument is found in some of the best laboratories.

Figure 31. Mounting Stand with Lamp

1 Journal of the Royal Microscopical Society, February, 1895, page 120.

21 - Lamps. This head may be suitably divided into:

1 - Alcohol or spirit lamps.

2 - Gas lamps.

3 - Electric lamps.

1 - ALCOHOL LAMPS, with stand and sand-bath (fig. 30) are excellent instruments on the work-table where illuminating gas is not available.

2 - GAS LAMPS: Bunsen burners are the usual forms. Some of Fletcher's patterns are also excellent for heating purposes; and Fletcher's "Instantaneous Water Heater" is an essential feature in the laboratory. This apparatus furnishes a continuous flow of hot water within a minute.

Figure 32 is a horizontal pattern and "the water passage is surrounded by a series of copper disks which are integral with it. A large surface is thus exposed to the flame, and is also allowed to become hot enough to admit of *direct flame contact*, thus greatly increasing its value as a heating surface. This construction also entirely does away with any dripping from condensation. The Water Heater is very compact, being only 14 inches wide and 6 high over all. It will deliver one quart of water per minute heated from 60° to 125°. There will be no smell from imperfect combustion unless too large a flame is used and the apparatus is driven beyond its capacity. It is very well calculated to withstand rough usage, and will not be injured by overheating if the gas is allowed to burn without a flow of water."[1]

Figure 33 is another form of heater which gives "instantly a continuous supply of water at any temperature, from boiling to lukewarm, free from the products of combustion, and suitable for all purposes. The gas supply should be ⅜- inch pipe. It will work equally well, but at a proportionately slower rate, with any gas supply, however

1 Buffalo Dental Manufacturing Company's Catalogue of Fletcher's Gas Furnaces and Laboratory Appliances, pages 43 and 44.

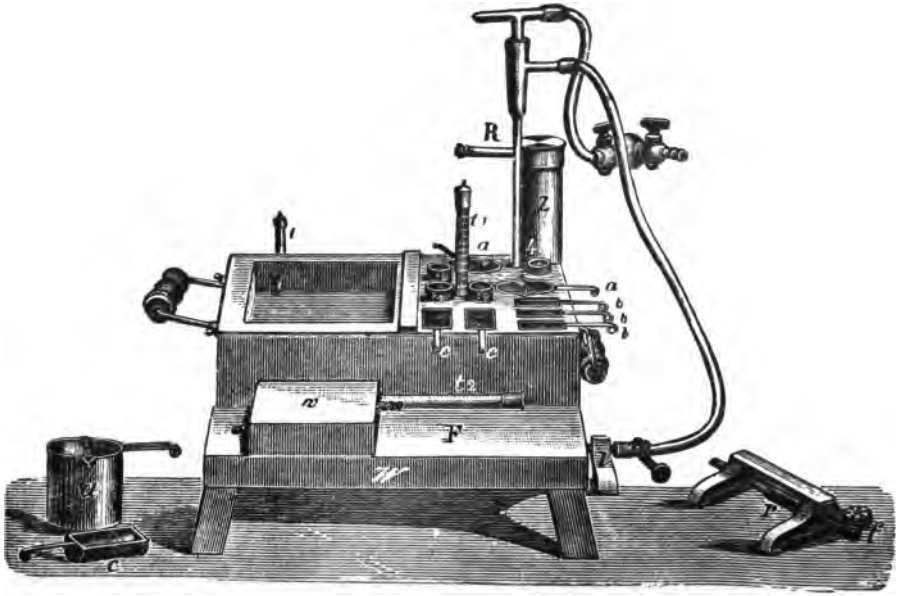
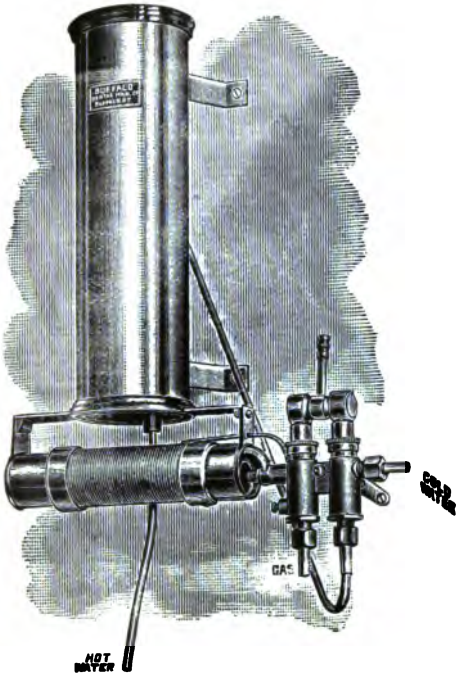
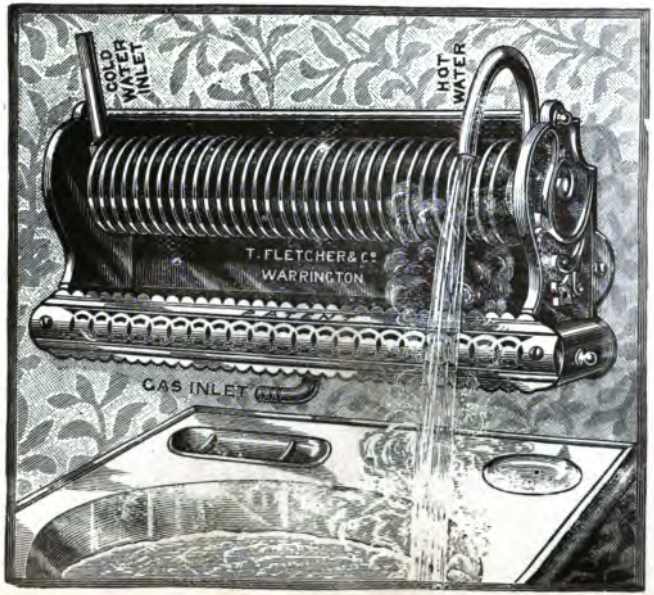


Figure 30½. R. Jung's Paraffin Bath.





Figures 32 and 33. Fletcher's Instantaneous Water Heaters.

Figure 35. Fletcher's Automatic Blow-Pipe.

Figure 36. Fletcher's Automatic Blow-Pipe.

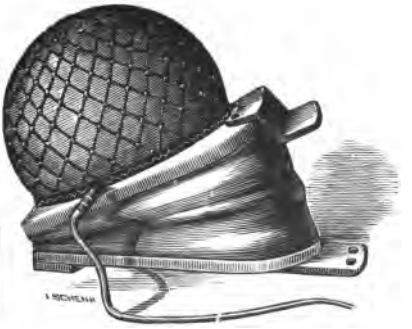
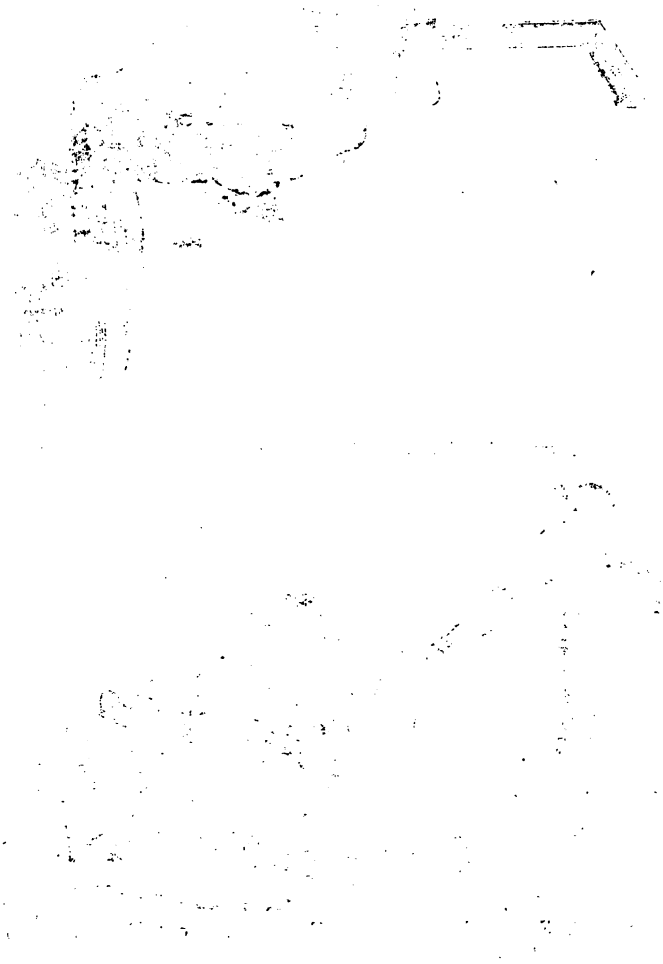


Figure 38. Foot Blower.



small. The speed at which the water runs rules its temperature. It will heat one pint of water per minute from 50° to 130° F., or it will boil 15 quarts per hour. A pilot light attachment, which keeps the water in the coil constantly warm, and automatically lights the burner when the gas is turned on," is a valuable addition to the instrument.[1]

Besides the water heater described above, the Buffalo Dental Manufacturing Company make several other heaters of special advantage in the Botanical Laboratory, some of which are illustrated in figures 34, 35, 36, 37, 38 and 39. These cuts represent so clearly the details of the heaters, very little need be said about them. Figure 34 is used for heating water, or other liquids, in large vessels, and the work is accomplished in an incredibly short space of time. It is simple in construction and not easily damaged.

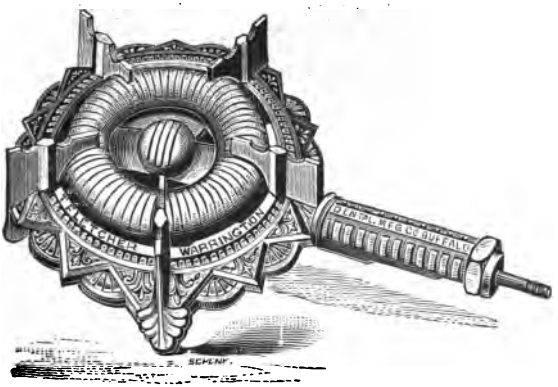


Figure 34. Fletcher's Radial Burner.

Figure 37. Fletcher's Self-Heating Soldering Bit.

Figures 35 and 36 will be found useful in the laboratory when it is desired to bend glass tubing into various shapes

1. Buffalo Dental Manufacturing Company's Catalogue. Page 44.

for adjustment to apparatus. The bellows, shown in figure 38, is used in connection with these blowpipes to supply the required air and blast. Figure 37 is a self-heating soldering bit, found very useful in mending portions of broken apparatus. A flow of gas passes through the handle, and a constant flame is thus kept on the copper. Figure 39 may be used as a substitute for 34, and it will serve all purposes for heating vessels of liquids. It will carry round glass flasks,

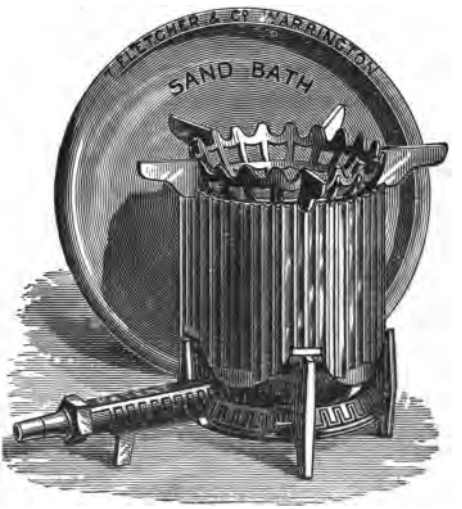


Figure 39. Fletcher's New Laboratory Burner.

flat-bottomed vessels and porcelain dishes, with perfect ease. The cylinder shown in the cut can be raised or lowered, so as to increase or diminish the distance between the bottom of the vessel and the flame. A sand bath also accompanies the heater.

Figure 40 is an excellent device for protecting the table while using the heaters above described. It consists of a board of asbestos, bordered with iron, and is an admirable non-conductor of heat. These asbestos pads will also be found useful in the laboratory for other purposes of protec-

tion—as, for instance, against chemicals—and will serve a good purpose to hold the wash bottles and furnish for them

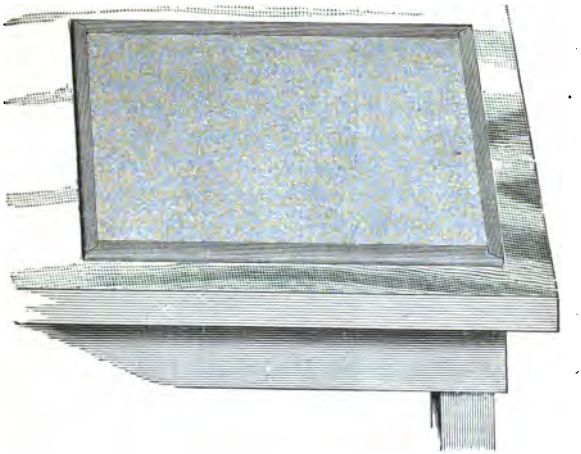


Figure 40. Asbestos Pad for protecting the Table during Blow-pipe Operations

a soft pad against the hard slate top, and thus diminish the danger of their breakage when placed on the table.

Care of Apparatus.—A delicate piece of apparatus, in good condition, is a most excellent assistant to the scientific worker; but if it is spoiled by careless handling, it will be a source of trouble ever afterwards. The microscope, when properly treated, will be a faithful servant for many long years, and will greatly aid the microscopist in solving many interesting problems which will be beyond his reach if the glasses and delicate parts have been grievously injured by bad management. Therefore, if the student desires to become a successful investigator in Nature's laboratory, he must learn a few rules in relation to the use of apparatus, and diligently endeavor to apply them.

1. Keep everything clean; eschew dust as one would the pestilence. If the lenses require cleaning, exercise the greatest care and gentleness in rubbing them, and use the

softest old linen, or, better still, the Japanese paper, which is free of particles of grit. Do not permit alcohol to touch the metal portions of the microscope, because it will dissolve the lacquer with which the metal is covered. In order to locate particles of dust on the lenses, twist the tube of the microscope while the eye is looking through the ocular, and with the other hand hold the ocular stationary. If the particles revolve with the tube, it is a sure evidence that the dust is on the objective, and, vice versa, on the ocular.

2. If the tubes or other portions of the instruments need oiling, moisten a clean cloth with the best watchmaker's oil and rub the parts. Take care that none of the oil reaches the lenses.

3. While using chemical reagents it will be wise to protect all metallic apparatus from fumes of acids.

4. Never focus *downwards*, for fear of injuring the objective lens or breaking the slide containing the object. Always focus *up*. In order to do this work correctly, bring the objective down by means of the rack and pinion movement, with the eye on a level with and looking across the stage; then it will be possible to lower the objective near the slide without bringing the two in contact. Now, with the eye looking through the ocular focus, up with the coarse adjustment until the image is brought into view and finish with the fine adjustment.

5. Begin work with the low powers of the microscope until an objective is found which will show the image of object distinctly in all its details. There is nothing to be gained in using a high power when a low power will do.

6. In using the immersion objectives, be certain to clean the lenses of all oil when the work is completed and before putting them away.

7. Chlorate of lime is an excellent absorbent of water, and when placed in the microscope case it will dry the atmosphere and thus prevent the rusting of the steel portions of the instrument.

CHAPTER V.

The Preparation of the Object for Cutting the Sections.

In preparing the specimen of the plant for permanent preservation on the slide, some definite order must be pursued; and the sequence usually adopted by microscopists may be stated in the following steps:

1. Eliminating the resinous matters from the plant, and softening, when required.
2. Staining (in some instances this may be deferred until the sections are cut).
3. Washing out, or infiltrating.
4. Dehydrating.
5. Clarification.
6. Saturation with parafine, or celloidin.
7. Imbedding.
8. Cutting the sections.
9. Fixation to the slip and removing the imbedding matter.
10. Mounting in Canada Balsam.

If the staining is deferred until the sections are cut, this operation will follow the fixation to the slip, and the stains must be alcoholic solutions, or otherwise the operation of dehydrating must be repeated after the staining is completed; or in other words, dehydration should be delayed until the staining is accomplished.

1. Extracting the Resinous Matter.— If the wood is green, it must be soaked several days in strong alcohol and afterwards immersed for ten days in water. Dry wood must be soaked for some hours in water, after which it is treated with alcohol and again immersed in water.

2. Staining.—The requisites for a good dye are:

(1) When in full strength its action should be *intense.*

(2) It should have *selective* properties rather than *diffusive.*

(3) It should penetrate well all portions of the specimen, staining equally the similar tissue near the surface and towards the centre of the section.

(4) The color should be permanent.

(5) It should not disintegrate the specimen, even though the process be prolonged.

All stains may be divided into two groups:

1. General stains.

2. Selective stains.

General stains act on all parts of the subject alike, while selective stains pick out one or more groups of fibre in the section, and, when properly applied, leave all other systems of the section unaffected.

To produce the best results with the selective stains, two methods have been proposed:

1. The staining must be stopped just as soon as the parts to be colored have received the maximum degree of dye and the rest of the section is beginning to be affected.

2. The section may be left in the selective stain and continued much beyond the first step, and the extra color eliminated by washing in alcohol, or dilute hydrochloric acid, in which the color stain is soluble.

In the process of coloring objects it is necessary, to get the best results, that the tissue should be dead, because living tissue will not, as a general rule, take up the stains so readily, but the living cells seem to repell the coloring matter. It has been found, therefore, advisable to first fix, or artificially kill the specimen before the sections have been cut; this is particularly true in reference to animal subjects, and in the case of those portions of plants where the centres of life will totally change unless they are quickly fixed in the form naturally assumed during the living period. The

process, stated in general terms, consists in so killing the subject as to prevent shrinkage or change in the elements. The entire mass, after being fixed, must be so hardened as to cause it to retain its natural shape and condition during all after treatment. After the fixing has been accomplished it is necessary to wash the specimen well in order to extract all the fixing agent. The character of this washing agent depends, of course, upon the kind of fixing agent used. For instance, if picric acid was used, the specimen must be washed with alcohol; if compounds of the heavy metals are used for the fixing agents, then the specimens must be washed in water. In other words, the washing must not leave the tissues in a soft condition so as to permit them to relax and fall from their normal shapes. The compounds of the heavy metals enter into such chemical combination with the tissues so as not only to harden and fix them, but a portion enters into union with the tissues and is insoluble in water. In the case of picric acid, however, alcohol, in which it is soluble, extracts it entirely, but during the washing the alcohol also hardens the tissues, and therefore the mass is left in a natural condition, although all the picric acid is eliminated. Some of the more important fixing agents may be enumerated as follows:

Chloroform.
Ether.
Alcohol.
Mercuric chloride.
Solution of chromic acid.
Picro-sulphuric acid.
Fleming's mixture.
Osmic acid.

Muller's solution (bichromate of potash 2 parts, sulphate of soda 1 part, and water 100 parts).

Merkel's solution ("Equal volumes of 1.400 solution of chromic acid and 1.400 solution of platinic chloride—$PtCl_4$. Objects should remain in it for several hours, or

even days, as it does not harden very rapidly. After washing out with alcohol of 50 per cent. to 70 per cent., objects stain excellently, notwithstanding the admixture of chromic acid.")[1]

Freezing.

The following are some of the more important nuclear stains by the first method, *i. e.*, by stopping the action of the dyes as soon as the color takes effect on the parts to be stained:

Methyl violet.
Methyl green.
Bismark brown.

The important nuclear stains giving the best results under the second method, *i. e.*, after prolonged staining and then washing out, are:

Anilin green.
Bismark brown.
Gentian violet.
Methyl violet.
Safranin.
Anilin red.
Magenta.

The stains suitable for coloring the protoplasm are:

Anilin blue.
Anilin green.
Picric acid.
Eosin.

One stain may be made to wash out another stain, at least to a partial degree. That is to say, when the section has been subjected for some time to the action of the coloring matter, which has a strong affinity for the nucleus, the protoplasm also becomes somewhat tinged with the dye. Now, if the section is transferred to a watch glass containing the stain suitable for coloring protoplasm the first color is eliminated by the second from all parts of the section

1 Lee's "Microtomist's Vade-Mecum." Page 30.

except the nucleus, and the section presents a compound coloring strikingly bringing out in strong contrast the nucleus and the protoplasm or ground.

The above stains are used for coloring sections. To stain in mass, as becomes necessary sometimes in plant histology, it is best to use cochineal or carmine. Hæmatoxylin is also valuable in the treatment of those specimens which are not affected by cochineal or carmine, particularly when the tissues have been placed in osmic or chromic preparations. This staining agent is also used for other histological purposes, which require its presence in the laboratory. Experience has demonstrated, however, that none of the old solutions of the stain hæmatoxylin is reliable, because of the presence of constituents in the solution in the condition of what is termed "unripe," "ripe" and "overripe." This makes the staining properties of hæmatoxylin very uncertain, and it becomes necessary to give the solution special treatment before placing in it the specimen to be stained. For this purpose Unna's method seems very good. It is given by Lee as follows:

"The spontaneous 'ripening' of hæmatoxylin solutions is due to the absorption by them of oxygen from the air, and the consequent oxidation of their hæmatoxylin into hæmatein. Unna has found (as Mayer had found before him) that a freshly prepared light red alum solution of hæmatoxylin can be instantly ripened—that is, converted into a dark blue solution of hæmatein of even much greater staining power than spontaneously ripened solutions—by the simple addition of a little neutralized peroxide of hydrogen. Such an instantaneously ripe solution may be conveniently prepared as follows: Make a solution of crystalized hæmatoxylin in alcohol, and another solution of about five times as much alum in water, and mix the two solutions in a test-tube. Then pour a little commercial peroxide of hydrogen solution into a watch glass, and add a crystal of soda. Remove the soda as soon as a blue litmus

paper remains blue in the solution, pour the solution into the test-tube and heat for a moment over a flame. The staining bath is now ripe, and must be used for staining *at once*, as a precipitation of the hæmatein begins to act in the moment the state of ripeness is attained." [1]

When carmine staining is resorted to, the "washing out" should be performed with a weak solution of hydrochloric acid (about 0.1 per cent.).

Mayer has devised an excellent stain of cochineal, which works well with any subject in the mass. It is stated as follows: "Cochineal, 5 grms.; chloride of calcium, 5 grms.; chloride of aluminium, 0.5 grm.; nitric acid of 1.20 specific gravity, 8 drops; 50 per cent. alcohol, 100 c.c. Powder the cochineal finely and rub up in a mortar with the salts, add the alcohol and acid, heat to boiling point, leave to cool, leave for some days standing, with frequent agitation, filter." [2]

Besides the coal-tar dyes mentioned above, there are also metalic stains which are found quite useful in coloring fibrous tissue and intercellular spaces. The chief of these are:

Nitrate of silver.

Chloride of gold.

The silver acts on the intercellular matters while the gold colors the cells. It is but proper to state, however, that the stains produced by these salts are not very permanent, but after a short time the specimens turn black and become unsightly for first-class histological work. The stains with gold chloride are very beautiful when first prepared and, if they could be made permanent, they would become very popular stains.

For more elaborate and detailed accounts of staining the student is referred to Lee's "Microtomist's Vade-Mecum," a most valuable and exhaustive work on the subject.

1 Lee's "Microtomist's Vade-Mecum." Pages 110 and 114.

2 Ibid.

Double or Compound Staining.—Beautiful results are secured by this process. The object of double-staining is to so differentiate the tissues as to bring out in striking contrast each group of fibre in the section and thus facilitate the study of them under the microscope. Double-staining is accomplished by the use of two dyes, one to stain the nucleus and the other to color the protoplasm or cell material outside the nuclear portions. The method of staining is the same as that used in simple staining, and the following represent some of the most important combinations for this purpose:

(1) Carmine-anilin blue. Clear with turpentine and mount in balsam.

(2) Carmine-metallic stains. (Use alum carmine with osmic acid. This carmine stain is highly commended by Lee. "To 50 or 60 grms. of water is added alum-carmine until the mixture is almost of a red rose color; about ten drops of a 0.02 solution of osmic acid are then added. The object should remain in the mixture for from twenty-four to forty-eight hours in the dark."[1])

(3) Hæmatoxylin-eosin. The sections should be very thoroughly washed after staining in the eosin and before transferring to the hæmatoxylin, since the presence of the former will precipitate the latter.

(4) Safranin-hæmatoxylin. This combination gives beautiful results. Stain first with hæmatoxylin, very slightly, then wash out with water followed by alcohol acidulated with hydrocloric acid, and stain for some hours with safranin.

(5) Safranin-gentian.

(6) Gentian violet-eosin.

(7) Methyl violet-eosin.

Resegotti has found by experiment that those stains which do not select the nucleus will wash out from the fibres the stains which are purely nuclear colors. He has classified them, therefore, into the following groups:

1 Lee's "Microtomist's Vade-Mecum."

Nuclear stains. Safranin, Dahlia, Methyl violet, Gentian violet, Magenta and Victoria blue.

The Secondary or non-nuclear stains are Methyl green, Iodine green, Congo, Orange, Acid fuchsin, Cyanin, Eosin, Methyl eosin, Bordeaux and Vesuvin.

It will be of importance to note that the properties possessed by the last group to wash out those found in the first list will enable the student to produce beautiful contrasts by chemical decoloration one of these colors produce on the other. It must be borne in mind, however, that the washing out process must not be prolonged, otherwise the color in the nucleus will be attacked and the double-staining will be destroyed. Transferring the sections to water will stop the elimination of the color; or they may be cleared and mounted at once. Clove oil may be used for the clearing agent, but it will also extract some more of the color, and this fact must be remembered or the section will present a faint contrast when mounted. Cedar oil, xylol and bergamot oil do not attack the stain and they may be used instead of the clove oil.

In the Journal of the Royal Microscopical Society for October, 1893, the following new process for double-staining vegetable tissue is given:

The sections are first decolored by eau-de-Javelle (hypochlorite of potash) and then left for a quarter of an hour in a concentrated alcoholic solution of cyanin, then washed with absolute alcohol and placed for a quarter of an hour in a 5 per cent. ammoniacal solution of congo-red. After washing in alcohol and mounting in xylol Canada balsam the sections present a magnificent double-staining; the liquefied membranes are colored an intense blue, and the cellulose membranes are dyed rose color or red.

3. Washing Out or Dehydrating.—The water found in all vegetable tissue is very deleterious not only to the free action of the imbedding material, but its presence also will cause the tissue to decay. Water has an

injurious effect on balsam, the mounting medium, causing it to precipitate and produce a cloudiness under the cover-glass of the slide.

Alcohol is the agent used for extracting the water from the tissue, and the method of procedure is as follows: The specimen is placed in rather diluted alcohol for a short time and then transferred to a vessel containing a stronger quantity of the spirit, and so on until 95 per cent. alcohol has been used. By pursuing this course of treatment there is slight danger of causing the sections to shrink. Some manipulators use absolute alcohol for the last washing, but this is not deemed necessary in most cases, since the small amount of water remaining in the specimen after floating for a while in 95 per cent. alcohol, will be removed by the clearing agent. One of the best appliances for producing this dehydration has already been described on page 50, viz.: Thoma's Dehydrating Apparatus.

In those cases where a fixing agent has been used, it is first important to remove this fixing agent before dehydrating is performed, unless alcohol is the removing agent, and then in that case it will partially dehydrate while it is removing the superflous fixing substance. Care must be exercised in performing this part of the operation not to prolong the dehydration too far, because the alcohol will cause the tissues to become hard, brittle, and eventually shrivel up into an unnatural form.

4. Clearing or Infiltrating.—When the dehydrating has been thoroughly accomplished, it is necessary to also remove the alcohol by replacing it with some agent which is readily miscible with the reagent to be used in the imbedding process, and this agent must also be anhydrous. This process is called *clearing*. It is very important, particularly in the case of delecate tissue, that the transition from the dehydrating agent to the clearing agent should be gradual. This is successfully accomplished by placing the clearing chemical under the dehydrating agent in some

suitable vessel, and then immersing the specimen in the top fluid, and it will settle by degrees into the thicker or lower liquid, and thus pass through strata of gradually increasing strength until the full strength of the clearing agent is reached. The alcohol is now drawn off and the specimen is ready to be transferred to the step in the process of mounting.

There are a number of clearing agents used by microscopists, some suitable for certain conditions under which others would not be capable of giving satisfactory results. The following are mentioned as among the most important chemicals for clearing the specimen of the dehydrating agent:

Oil of cedar-wood.
Oil of cloves.
Xylol.
Naphtha.
Oil of bergamot.
Toluol.
Oil or essence of turpentine.
Creosote.
Benzole.
Chloroform.
Anilin oil.
Carbolic acid (for clearing quickly objects poorly dehydrated).
Oil of origanum.
Oil of sandal wood.

Oil of cedar must be thin in consistency and of a light yellow color to give the best results. This oil evaporates slowly and mixes well with balsam dissolved in chloroform. It clears specimens very well and readily which have been dehydrated in 95 per cent. alcohol. Mr. Lee says, in reference to this oil: "A laboratory equipped with cedar oil and origanum oil is fully equipped for all possible cases (the origanum oil being used merely to take the place of cedar wood oil for the special case of celloidin sections)." Cedar

oil clears tissues stained in anilin dyes without extracting the color.

Oil of cloves is a very good clearing agent for certain kinds of work where minute dissection is resorted to. The specimen, however, cleared in it is very apt to become brittle after a short soaking in the oil, and this feature, in many cases, will be a decided disadvantage. The tendency to form minute drops over the slide gives it a value for minute dissection. Mixing it with oil of bergamot will reduce its tendency to produce brittleness in the tissues. It is an excellent clearer, and, when properly used, will be found to be a valuable addition to the laboratory equipment.

Benzole, naptha, chloroform and xylol are so volatile it will be quite difficult to use them with advantage in infiltrating operations, but they will be useful in clearing the sections after they are cut.

Oil of bergamot does not destroy the anilin colors placed in the tissues, and it has the additional valuable property of clearing the specimens very quickly which have been dehydrated in 95 per cent. alcohol.

Oil of turpentine is only valuable for clearing specimens after they have been cut in paraffin. Its disadvantage consists in the shrinkage it gives the cells, and in some instances, where alcohol is present, altering even the structure.

Anilin oil is a strong clearing agent, and it also acts quite well as a dehydrating agent. It will extract water from specimens which have been treated with 70 per cent. alcohol. For certain kinds of work it will be found exceedingly valuable—particularly for preparing specimens for paraffin imbedding. Unless this agent is removed by soaking for several hours in chloroform, however, the sections will turn yellow after a while.

Carbolic acid is another dehydrating, as well as clearing, chemical, and is best used in a concentrated solution in alcohol. Care should be exercised, however, in its use, because it will produce a rapid change in the form of the

fibre when the sections are mounted in balsam. It is valuable for celloidin imbedding.

Oil of origanum. This oil is valuable, since it will clear specimens which have been stained in anilin dyes without affecting the colors to any serious degree. It does not volatilize easily and rapidly, and is unchanged when exposed to the action of the light. The odor is not disagreeable. Celloidin sections are quickly cleared by it without dissolving the celloidin.

The other agents mentioned in the list are less valuable than those described, and are not absolutely necessary in the equipment of the laboratory.

CHAPTER VI.

Imbedding Preparatory to Cutting the Sections.

The clearing process described in the preceding chapter is also an infiltrating or penetrating method preliminary to the imbedding operation. This clearing work is necessary before the object is imbedded in paraffin, because otherwise the paraffin would refuse to enter thoroughly into all portions of the mass, so that cutting with the microtome would become difficult if not impossible. It is, therefore, important that the infiltrating work be correctly and thoroughly done, in order to secure satisfactory results with the imbedding material. The oil used in the infiltrating process must penetrate all portions of the mass, and the water must be entirely eliminated, and the clearing or infiltrating medium must also be a good solvent of the paraffin used in the imbedding work. With these simple facts well borne in mind, the student will proceed as follows to complete the imbedding of the specimen preparatory to cutting the sections. The steps given are those usually taken by the best microscopists in this country and abroad, and are very strongly recommended by Lee and others, and much of the information below has been condensed from the excellent work "The Microtomist's Vade-Mecum," to which the student is referred for a full and detailed description of the methods in use for the imbedding and cutting of sections.

5. Imbedding in Paraffin.—Melted paraffin is substituted for the infiltrated substance by immersing the specimen in a vessel containing paraffin of the lowest melting point. The specimen is kept in the imbedding mass until it becomes thoroughly impregnated with it. The paraffin should be placed in one of the standard water baths

and kept just at the melting point. Duration of bath will depend on the nature of the specimen and the character of the clearing agent employed. Prolonged heating at any time is injurious to many forms of vegetation, and it should therefore be born in mind that the employment of paraffin of low melting point and the reduction in the degree of heat, shortening as much as possible the time of the bath, will secure the best results.

After saturation has been accomplished the specimen is transferred to the melted paraffin contained in the imbedding box described on page 54 and illustrated in figure 28, or in some similar box for the purpose, arranged as desired, and then cooled with water as soon as possible. Care should be exercised not to permit the water to enter into the warm paraffin, because if it does, the mass will at once become filled with holes and thus be injured for yielding good results in the microtome. As soon as cooled, the mass is shaped into a rectangular form, so that when placed in the microtome one face of the mass will be square with the knife and the opposite edge parallel with it. The microtome knife must not be moistened with liquid of any kind, but kept dry during the entire time of cutting the sections, so that rolling of the paraffin will be largely prevented. The temperature of the room should be made to sustain a certain relationship to that of the boiling point of the paraffin. In winter, when the melting point of the paraffin is 45° C., the temperature of the laboratory should be between 15° and 17° C.; in summer the relationship should be, for paraffin, 48° and 22° for the laboratory. The temperature of the paraffin, in winter, may be controlled by means of a reflector lamp, so placed as to have the heat rays reflected on the sections as they are being cut; in the summer, this lamp may be replaced by a block of ice, with the reflector projecting the cool rays upon the mass of paraffin; or the room may be regulated in temperature by the adjustment of the window fixtures.

If it is desirable to produce in the cutting what is termed "ribbon-sections," it will be best to have in the laboratory one of the microtomes made for that purpose, such as Minot's automatic microtome, or any other of the same character; but it is possible to make these ribbons with the judicious use of the knife on any of the ordinary standard microtomes. The following hints are given to aid the student in accomplishing this end:

1. The edge of the section is held down by means of a camel's hair pencil until contact is made with the next section and the heat from the lamp causes it to cohere.

2. The block of paraffin must be pared down very close to the object and so cut as to present a square edge to the knife. The cutting must be rapid, so that the blows of the knife will slightly heat the paraffin, and thus cause the edges of the sections to cohere and form the ribbon.

3. The use of what is known as "the section stretcher," which is so placed as to rest on the upper surface of the knife, and thus hold the sections in a flat position and in contact with each other best calculated to form the chain.

In the case of those specimens which are so brittle, or the parts of which are so slightly cohering as that separation takes place during the cutting, the following method of procedure is recommended: A bottle of collodion is placed convenient to the microtome, so that a brush may be dipped in it and the surface of the paraffin covered with a thin coating of the collodion as each section is cut. The collodion must be of such a consistency as to dry quickly without leaving a polished residue on the section. This coating must be made very thin, or the results will not be satisfactory. Repeat the operation after cutting each section, and the collodion will be found to sink into the specimen and upon drying will hold the parts together. When the section is taken from the knife it should be transferred to the slide with the collodion surface downwards, in contact with the slide, which has been previously prepared with Schalli-

baum's fixative. ("One part of collodion is shaken up with three to four volumes, according to the consistency of the collodion, of clove oil or lavender oil. This should give a clear solution. A little is spread on a slide with a small brush. After arranging the sections on the prepared surface, warm over a water bath, gently, until the clove oil has evaporated—five to ten minutes."[1])

For cutting ribbons Adam Sedgwick proposes the use of softer paraffin around the block of harder paraffin in which the specimen is imbedded. After the imbedding has been accomplished, the block is dipped in softer melted paraffin and the surplus is cut off, leaving only a thin layer of the coating on the front and rear faces of the block. When the cutting begins this soft paraffin will cause the sections to unite into the ribbon form.

In the labratory there should be found two grades of paraffin. One melting at a temperature of 45° to 46° C. and a hard paraffin melting at a temperature 55° to 56° C. The microscopist can then mix these two together in proportions to suit the condition of temperature in the room. It must be remembered that the harder the paraffin the thinner may the sections be cut, but there is stronger liability to roll. It is best, therefore, to mix the two kinds in such proportions to produce a paraffin which will give a melting point of 50° to 55° C. This will have to be determined by experiment, but ordinarily the combination may be secured by mixing in the proportion of 20:9.

Plant tissues vary so much it is necessary to use great care in their treatment to prevent shrinkage while passing through the several liquids preparatory to imbedding for paraffin cutting. In the case of those specimens which contain much meristemic and watery thin-walled tissue, the following procedure is recommended by Dr. J. W. Moll in the Botanical Gazette for January, 1888: The dehydrating of the specimen was accomplished by using a solution of chromic

1 Lee's "Microtomist's Vade-Mecum. Page 212.

acid and 20 per cent., 35 per cent., 50 per cent., 75 per cent., and 90 per cent. alcohols. The chromic acid acted on the protoplasm so as to fix it and macerate the cellulose, and thus permit the alcohol to permeate more freely. The dehydration must be done by slow degrees, so it is best to permit the specimen to remain in the several alcohols for twelve to twenty-four hours, depending upon the size of the specimens under treatment. Passing from the alcohols, the plant is placed in a solution of equal parts of turpentine and paraffin. This solution is now raised from a temperature of 20° C. to 45° C. The specimens are then placed in melted paraffin, which is retained at a temperature of 50°. It will take from one to two hours to permeate the specimens.

Mr. Gustav Mann, in the Transactions and Proceedings of the Botanical Society of Edinburgh,[1] thus describes a method of preparing vegetable tissue for paraffin imbedding: "Requisites: (I) Picro-corrosive alcohol. Heat absolute alcohol to 50° C., saturate with picric acid, and then add bichloride of mercury to saturation. When cool decant. This solution may be made in quantity and kept. (II) Absolute alcohol. (III) Chloroform-alcohol chloroform and absolute alcohol mixed in equal parts. (IV) Chloroform. (V) Solid paraffin, melting point 46°–50° C. (VI) Short, wide mouth bottles. (VII) Best cork stoppers, two for each bottle; the one fitted with a piece of glass tubing 1 cm. in diameter and 3 cm. long. (VIII) Number of glass rods drawn out into fine points, as one must avoid bringing metal instruments in contact with the picro-corrosive fluid.

"Method: A. The fixing and hardening of tissues: Place the tissue in at least fifty times its bulk of the picro-corrosive alcohol. Leave small objects (up to 1 cubic cm.) for twenty-four hours, larger objects for forty-eight hours and upwards in the fluid. Keep the bottle well corked.

"B. The replacement of the picro-corrosive alcohol by

[1] Journal of the Royal Microscopical Society. Page 686, 1891.

pure absolute alcohol. (1) Pour off the hardening fluid till the tissues are just covered. Add absolute alcohol, according to the size of the tissue, in 1–10 drops every ten minutes till the tissue is again in fifty times its bulk of fluid. After each addition move the bottle very gently to allow the added alcohol to mix with the hardening fluid. Leave the tissue in this diluted mixture for twenty-four hours. In no case should this process be hurried, or strong diffusion currents will be set up, and the protoplasmic contents of the cell separate from the cell-wall. (2) Pour off the fluid till the tissue is just covered, and add absolute alcohol to the original bulk. Move about the bottle every three or four hours. Most of the picro-corrosive material will thus be extracted after twenty-four hours. (3) Draw the fluid off rapidly by means of a pipette, and add absolute alcohol up to half the original bulk. Any drying of the tissue must be carefully guarded against. Leave for twenty-four hours and repeat the process.

"C. The replacement of the alcohol by chloroform: (1) Pass, by means of a pipette, the chloroform-alcohol mixture to the bottom of the vessel, when the tissue will float on the mixture, remove then the supurfluous alcohol by a pipette, leaving only enough to cover the tissue. (2) When the tissue has sunk in the chloroform-alcohol mixture, introduce by a pipette pure chloroform, on which the tissue will float; the fluid above the tissue is removed by a pipette. After twenty-four hours the tissue may or may not have sunk in the chloroform; if not, it may be induced to do so by heating the chloroform to 20° C. (not higher); if this fail, a little sulphuric ether may be added. After the tissue has sunk leave for twenty-four hours. (3) Place a fresh supply of chloroform at the bottom of the vessel (50 times the bulk of the tissue), and if there is a distinct line of demarcation between the newly-added and the old chloroform, the upper layer should be removed by a pipette.

"D. The replacement of the chloroform by paraffin: (1)

Place the tissue in a warm chamber, heated to 25° C.; add solid paraffin in pieces up to the size of a small pea. After each piece has dissolved, the bottle has to be moved about very gently to hasten the mixing of the paraffin, which will be in the upper layers, with the chloroform. Continue until no more paraffin dissolves. Tissue which did not sink in pure chloroform will always sink as soon as paraffin is added. (2) Place the tissue in a warm chamber heated to 30° C. for twenty-four hours. (3) Place the tissue in a warm chamber heated to the melting point of the paraffin (46° C.), and after six hours replace the ordinary cork stopper (which up to this stage has always to be employed) by a perforated one. This method is adopted to insure a gradual giving off of the chloroform, for I find that, if the latter be driven off rapidly, a good deal of shrinkage always results. When all the cloroform has evaporated, i. e. if after shaking the bottle gently one is unable to detect, by smelling, the faintest trace of chloroform, then the tissue is ready for sectioning. (4) The tissues should not be exposed longer than just necessary to the temperature of melted paraffin, but should be imbedded by means of Leuckart's type-metal box."

Herr F. Rosen gives the following method for imbedding vegetable tissue so as to produce the least contraction possible:[1] "The objects, thoroughly dehydrated in absolute alcohol, are successively transferred (1) to a mixture of equal parts of absolute alcohol and bergamot oil; (2) to pure bergamot oil; (3) to a mixture of equal parts of bergamot oil and paraffin; (4) to paraffin with melting point of 45° C.; (5) to paraffin with melting point 56°-58° C. for twenty-four hours.

In stages (3) and (4) the fluids should be kept at 48°, in stage (5), at 60°. In order to make the sections adhere to the slide, they are placed, while still in paraffin, on a drop of fluid which can be evaporated completely, such as

1 Roy. Mic. Soc. Journal, Oct., 1894. Page 632.

distilled water, or 50 per cent. pure alcohol. The paraffin is dissolved out by xylol. The alcohol must be completely evaporated before the treatment with xylol, otherwise the sections will get free."

Imbedding in Celloidin or Collodion.—"Among the very various methods of imbedding that have been proposed, two are pre-eminently important—the paraffin method for small objects, and the celloidin or collodion method for large objects.

"The subject of the respective merits of paraffin and celloidin still affords matter for discussion to some persons. The case, however, seems to be a very simple one. Celloidin does not afford, by a long way, the thinnest sections that are obtainable with small objects. For such objects it is, therefore, not equal to the demands made by modern minute anatomy, and paraffin must be taken. On the other hand, paraffin (as at present employed) will only cut very thin sections with small objects; with objects of 7 millimetres diameter, you cannot get, with paraffin, thinner sections than you can with celloidin; and if you try to cut, in paraffin, objects of somewhat greater size, 10 mm. and upwards, it will probably happen that you will not get perfect sections at all, blocks of paraffin this size having a tendency to split under the impact of the knife; so that for large objects, celloidin gives better results." [1]

Celloidin is a preparation of pure pyroxylin (nitrocellulose), manufactured in Germany and sent out in the form of tablets of a tough, gelatinous consistency. It is soluble in ether and absolute alcohol. Imbedding in collodion was first introduced by Duval, in 1879, a afterwards improved on by the introduction of celloidin. The method recommended in the use of celloidin is as follows:

The object is first thoroughly dehydrated in absolute alcohol, and then soaked in ether until saturated, or in a mixture of ether and absolute alcohol (10:1). From this

1 Lee's "Microtomist's Vade-Mecum."

solution it is transferred to solutions of celloidin, of various degrees of consistency, beginning with a thin solution and passing from thicker to thicker until the specimen has been thoroughly impregnated with the celloidin. Busse proposes using three solutions. The first consisting of 10 parts, by weight, of celloidin and 150 parts of ether and alcohol mixture; the second, consisting of 10 parts of celloidin and 105 parts of the ether-alcohol mixture; and the third consisting of 10 parts of celloidin and 80 parts of ether-alcohol mixture. The object must remain in the first bath for a length of time sufficient to permit of thorough penetration. To accomplish this may require several days. After soaking in the last solution, the specimen is imbedded in the celloidin by either one of the following methods:

1. The imbedding material is moulded in the paper box, or metal box mentioned on page 54. The bottom of the box is covered with a layer of the thick celloidin and allowed to dry by evaporation; another layer is placed on this and allowed to dry, and so on until a sufficiently thick coating is secured in the bottom of the box. The object is now arranged on this coating of celloidin and fresh solution added until the object is entirely covered with the imbedding mass to the depth desired. After the filling has been completed, the box is placed under a bell-glass resting on a ground glass plate and the celloidin allowed to slowly dry in the alcohol atmosphere generated from the block of imbedding material. When sufficiently hard to resist the pressure from the ball of the finger, the paper or metal box is removed and the mass is immersed in chloroform where, in a short time, it assumes the form of a hard coagulated body resembling wax, and beautifully transparent. After coming from the chloroform it is placed in a vessel containing white oil of thyme, or similar oil, where it will assume, in a few hours, the appearance of a clear glass-like mass.[1] It is best to use the chloroform immersion for small

1 American Naturalist, XXVI, 1892. Page 80.

objects, but for larger bodies it is recommended by many microscopists to use alcohol of 85°. Absolute alcohol must not be brought in contact with the imbedding material because it dissolves celloidin. In the 85° alcohol solution the block of imbedding material must remain until it assumes the consistency required for good work in the microtome. This may take several days. The vessel containing the alcohol must not be closely corked. After the mass of celloidin has been well hardened it can be preserved for a long time in 70° alcohol.

While the block of celloidin is being cut, it must be kept moist with 70° alcohol, since it dries very rapidly. If, however, the oil of thyme treatment has been resorted to, the knife of the microtome may be lubricated with this oil, thus making it possible to cut thinner sections. Moreover, the oil does not evaporate quickly like the alcohol, and the operation may be stopped for some time without serious detriment to the celloidin mass.

If the object has not been stained in mass, it may be difficult to distinguish it in the mass of transparent celloidin. Under this condition it will be best to slightly color the celloidin with picro acid or other similar stains.

When submitted to the pressure of the microtome clamps the celloidin yields slightly, and there is danger of distorting the object and thus destroying its histological value. To avoid this trouble, the block of celloidin, after being properly hardened, is mounted on a piece of wood suitably shaped for being grasped by the microtome. A coat of the thick solution of celloidin is spread over the surface of the wooden block and allowed to dry. The under surface of the celloidin block is trimmed to expose fresh surface and a solution of the celloidin smeared over it. The two surfaces thus prepared are pressed together and then placed in a solution of 70° alcohol to soak for a few hours, in order that the point of contact may become hardened. The

wooden block is now clamped in the microtome and the sections cut in the usual way.

2. This method consists in building up the block of celloidin on the wooden support from the start. The surface of the wood is slightly roughened so that the material will stick securely, and a layer of the thick celloidin is spread over it and permitted to dry, and then another layer is added, and the process repeated until the foundation is built up high enough, when the object is put into position and the celloidin placed around and over it until it is entirely covered with the imbedding mass. The hardening of the celloidin and after treatment are the same as used in the first method.

Herr O. Jelinek[1] recommends the use of the new insulating material called stabilite in the place of the wooden support for celloidin imbedding. Its advantage consists in its insolubility in water or alcohol (wood and cork are slightly soluble in alcohol, giving up coloring matters and tannic acid); it can be cut by a saw into any shape desired; it is firm and will withstand the pressure of the microtome; it is cheap; it may be readily written on with pencil or ink, which are not easily effaced while under treatment; it is not hygroscopic and will quickly sink in alcohol, thus bringing the imbedding mass in thorough contact with the hardening solution; it is not attacked by dilute hydrocloric and sulphuric acids.

It is important, says Dr. A. Elsching,[2] that celloidin solutions should be free from air and water. To effectually deprive celloidin of water, the tablets should be cut up into little blocks, the sides of which are not bigger than 5 mm. These are placed between folds of blotting-paper, and first allowed to dry at the room temperature, and then desiccated in an incubator. At this stage they should be of a yellowish

1 Zeitscher. f. wiss. Mikr., XI., 1894. Page 237.

2 Journal Royal Mic. Soc. June, 1894. Page 404. And Zeitschr. f. wiss. Mikr., 1893. Page 445-6.

hue and of horny consistence. Absolute alcohol may be easily obtained entirely free of water, by repeated treating with freshly dried copper sulphate. The dried cubes are placed in a narrow-necked bottle, with air-tight stopper, and form a layer not exceeding one-fourth the volume of the bottle. The celloidin is then just covered with the absolute alcohol, and allowed to stand for about twenty-four hours, after which the ether is poured in. In a very short time the celloidin is all dissolved, and thus, as no stirring is required, the solution is kept free from air-bubbles.

Freezing in the place of imbedding is often resorted to by microscopists in cutting sections from soft tisues, and excellent results are generally secured. In using this method, however, care must be exercised to prevent, as far as possible, the formation of chrystals in the cells of the delicate tissues so that rupture of the cells will take place. This trouble is greatly overcome by the use of some gummy substance which will not form chrystals when the specimen is frozen. The gum is infiltrated into the specimen. It is recommended in the Journal of the Royal Microscopical Society that when the specimen is soaked in oil of aniseed for some hours—twelve to twenty-four—the freezing may be safely accomplished without detriment to the tissues. The oil is dissolved out of the sections by the use of alcohol. The freezing is successfully done by Bausch & Lomb's apparatus, illustrated and described on page 52.

CHAPTER VII.

Cutting the Section, Fixation to the Slip and Mounting.

For the accomplishment of first-class work with the microtome, the knife must be kept in the best condition possible. It must be ground so keen that a hair resting on the edge will be severed. Messrs. Bausch & Lomb, of Rochester, N. Y., give the following excellent instruction for obtaining this condition of the knife:

"The requisites for good work in microtomy are, aside from the manipulative skill and the preparation of the specimen, a perfect working microtome and a good knife. The latter particularly, for, if the cutting edge is not in the best condition, no good sections can be secured; we have had so many complaints from this source and for the reason that so few know the real *modus operandi* to obtain a keen cutting edge, that we append these instructions how to proceed to sharpen a knife and keep it in good condition.

"We recommend, to commence with, the yellow Belgian hone, for the reason that it is the best obtainable; in using it cover with palm oil soap and moisten freely with clean water so that a lather will be formed, which must be kept up during the entire time the knife is being sharpened. This soap has the advantage over the ordinary oils in use for honing, in that the pores of the stone remain open, whereby quicker sharpening as well as a better edge and easier cleaning of the knife are the result. If a microtome knife is very dull, or perhaps has small nicks in it, it is very essential to first sharpen it on a yellow stone and then draw it several times over a blue stone in order to obtain an exceedingly keen edge; with the blue stone is used a softer stone called 'rubber' which is necessary, for, after

the blue stone has been moistened with water, it is used to grind down the scum.

Figure 41.
Microtome Knife.

"To begin with, the knife is sharpened on the concave side until a fine thread is formed on the cutting edge, which can easily be seen by drawing the knife across the finger nail; when this thread becomes visible along the entire cutting edge, the knife is passed without any pressure over the already prepared stone, but in such a manner that the entire edge is always towards the front as shown in the cut, Fig. 42. It is necessary that the mentioned thread be formed on the concave side only, in order that the amount ground off on the flat side be as little as possible. Should the ground surface on the lower flat side be too large, it will no longer be possible to obtain a perfect cut, and the knife must be, first of all, reground on the flat surface. As soon as the thread appears, the knife is drawn equally to the right and left in the indicated directions, without executing the least pressure of the hand on the stone. After a uniform keenness has been obtained by light and continued sharpening, its quality can be judged by passing the edge of the knife lightly over the moistened tip of the thumb, and if the feeling that the knife will enter the skin be apparent, it shows that a sufficient keenness has been obtained on the stone, and that its perfection can only be attained by passing it over the strop. The knife is then carefully cleaned with an old linen cloth

held between the thumb and index finger, but in such a manner that this cloth does not touch the edge. After cleaning it, it is lightly and carefully drawn across the strop and without pressure of the hand (see figure 43).

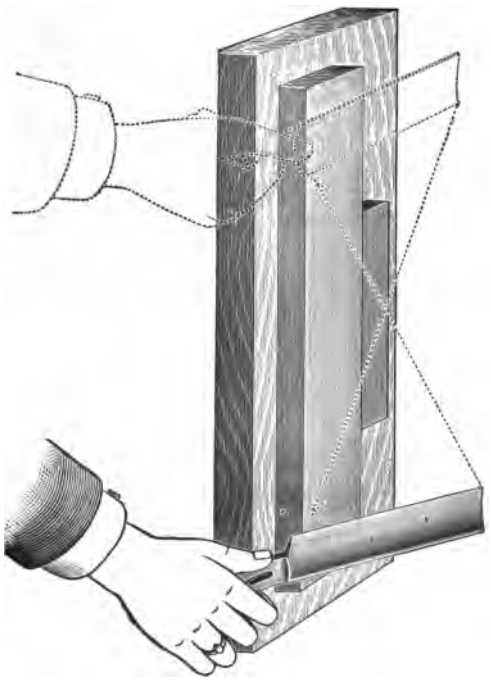


Figure 42. Sharpening Knife on Stone.

If the knife will now cut a hair freely alone the entire edge, it has attained the requisite keenness of a microtome knife, and after it has again been wiped off with a soft chamois skin, it is ready for cutting. It is to be suggested that the strop is slightly covered with oxide of iron, and rest on a flat and firm support. Before each cutting of the preparations, the knife should be passed over the strop." [1]

1 "Microscopes and Accessories," by Bausch & Lomb Optical Co. Page 82.

Having put the knife in the keen condition as above described, the specimen to be cut is clamped in the carriage of the microtome, and by means of the micrometer screw, it is raised in the plane of the knife and the sections made. The degree of movement of this micrometer screw will determine the thickness of the sections. The milled head

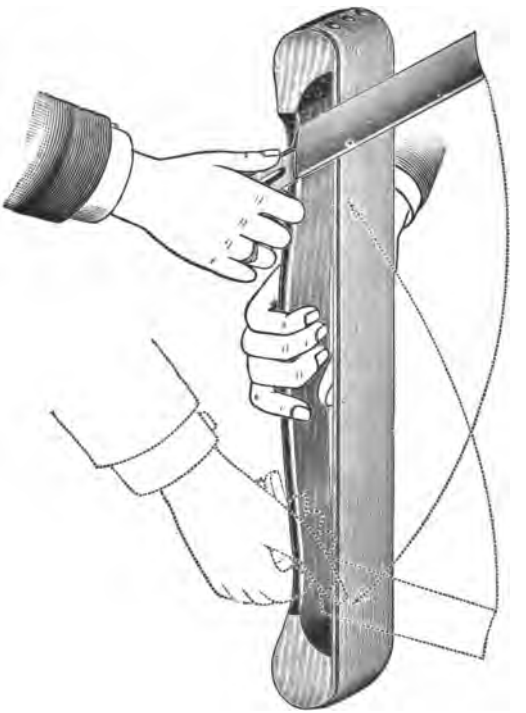


Figure 43. Stropping Knife.

attachment to this screw is graduated into millimeters, so that the amount of elevation of the specimen can be accurately measured.

Fixing the Section to the Slip of Glass.—After the sections have been properly cut without or with

the imbedding mass, described in a previous chapter, and it is proposed to mount them on the glass slip for permanent preservation in accordance with a certain order of arrangement, it is necessary to resort to some method of fastening or fixing them on the slip so that they will adhere to the glass, and not change their positions during the treatment to be hereafter described in staining and mounting.

If the specimen was stained in bulk, then mounted in paraffin and the sections cut with the imbedding mass attached, the following method is recommended by Schallibaum to cause the sections to adhere to the slip while the imbedding mass is being removed, and such other manipulations as are necessary to complete the mount.

This treatment is called the Collodion method, and consists in spreading a thin coating of the following preparation over the glass slip, after which the sections are arranged on it and the slip is warmed over a water bath until the oil of cloves is evaporated, which will take about ten minutes. The sections will then adhere so firmly that the after treatment will not displace them. The solution is made as follows: "One part of collodion is shaken up with three to four volumes (according to the consistency of the collodion) of oil of cloves or lavender oil. This should give a clear solution." [1]

As has been already stated, this method is not safe to use in case the staining is to be accomplished after the sections have been fixed to the slide, because it is found difficult to cause the flattening out of folded sections, and the collodion also is stained with most anilin dyes. It is, therefore, best to use, in preference, Mayer's method. This is an albumen preparation, and consists of solution made of "white of egg, 50 c.c.; glycerine, 50 c.c.; salicylate of soda, one grm. Shake them well together, and filter into a clean bottle. I find it convenient to beat up the white of egg with a little water before adding the glycerine and filter-

1 Lee's "Microtomist's Vade-Mecum." Page 210, 3d Edition.

ing; the salicylate should be dissolved in water in the first instance. According to my experience, carbolic acid is perfectly efficient as a preservative, but it is not to be recommended, because it precipitates a great deal of the albumen."[1] A thin coating of this solution is placed on the slip, and after the sections are arranged on it the slip is warmed in the water bath until the paraffin melts, when the albumen is dissolved and carried off with it. The sections may now be treated with any of the aqueous and alcohol stains, and they will not be disturbed from the position they occupy on the slip. The paraffin may also be removed in the cold condition, by treating it in a vessel containing toluol or xylol, but afterwards the slip must be thoroughly washed in alcohol in order to eliminate all traces of glycerine, or otherwise cloudiness will be produced in the after treatment.

If it is found that the staining will also affect the albumen, it becomes necessary to use another method for fixing the sections to the slide. This treatment consists in moistening the surface of the glass with alcohol or water, and after the sections are arranged with a brush, the slip is warmed gently, when the adhesion will be sufficiently strong to permit of mounting, if great care is exercised. The cover-glass is placed on and the paraffin is dissolved out by inserting under the cover Canada balsam dissolved in xylol, in equal parts.

Sections which are cut in celloidin or collodion imbedding masses, must be fixed to the slip by means of Summer's ether vapor method. The sections are first placed in 95 per cent. alcohol for a few minutes, and then arranged on the slip; sulphuric ether *vapor* is poured on the slip from a bottle half full of the liquid ether. The celloidin will become softened and transparent. The slip is now transferred to a vessel containing 95 per cent. alcohol, when it will be found that the sections are firmly fastened to the glass.[2]

1 Lee's "Microtomist's Vade-Mecum." Page 215.

2 American Monthly Microscopical Journal, 1887. Page 73.

Mounting in Canada Balsam.—After the sections are properly stained, dehydrated and fixed to the slip, it becomes necessary to mount them in some medium which has preservative properties, otherwise decay will set in, and in time, the sections will be ruined. One of the best mounting media is Canada balsam dissolved in either xylol, benzole, chloroform or turpentine, because of its refractive properties and the ease with which it is manipulated, and its valuable preserving powers.

In the use of this medium, it is absolutely necessary that the specimens be thoroughly dehydrated, or a cloudiness is produced of watery vapor, and the slide will be sadly ruined until the cover-glass is removed and a new mount is made.

The glass generally used for holding the object is made of the best white crown glass, measuring 76x26 mm. (3x1 inches), the edges of which are ground. The cover-glasses are thin circles cut to dimensions of 12, 15, 18, 21 and 24 mm. Square cover-glasses of the same dimensions can also be purchased from dealers, when such shapes are desired. The square forms are not suitable, however, when the manipulator wishes to use the turn-table to form a ring before and after placing on the cover-glass, and it is necessary to complete the mounting and sealing without the turn-table when the square forms are used. The thickness of the cover-glasses varies from 0.15 to 0.22 mm. When the immersion objective is used, it becomes desirable to know this thickness.

Of course it is important that these glass slips and covers should be scrupulously clean, free from all films of grease and other extraneous matters, before the sections are fixed, otherwise serious trouble awaits the operator. The cementing ring will fail to attach firmly to the surface, and the mount will present a slovenly appearance. For recipes suitable for cleaning the glasses, the student is referred to the pages at the close of this volume.

The mode of procedure for making the complete mount is as follows: If the sections do not require a previously prepared ring for the glass-cover, place the slip on the turn-table and centre accurately. By means of a brush or needle put a drop, or more if required, of Canada balsam on the sections, and the proper size cover-glass is grasped between the forceps and is gently lowered to its place over the sections and pressed down until the balsam is entirely spread out, filling up all the space between the cover and the slip. To accomplish this part of the work to the best advantage, in order that no air bubbles may give trouble after the mount is completed, the edge of the glass cover opposite to the forceps is first brought into contact with the slip and then it is gradually lowered until nearly in contact all around, when the forceps are removed and the circle is permitted to fall gently into place. A spring clip is now placed on so that all superflous balsam is driven out, as well as most, if not all, of the air bubbles. If a few very small air bubbles remain, these will pass out when the balsam begins to thicken in the course of a few hours.

After seasoning for two or three days, or longer if required, to well harden the balsam between the circle and the slip, the mount is placed on the turn-table again and, by means of a scapel, the balsam remaining outside of the cover is carefully scraped off as the table is slowly turned. A cloth, moistened in benzole, is rubbed over those portions of the glass still containing films of balsam, extra care being taken, however, to avoid displacing the cover-glass and dissolving the hardened balsam under its edge which cements it to the glass slip. All balsam must be removed and the benzole dried off to prevent the finishing ring from running when it is applied. When this cleaning is thoroughly completed, the cover-glass must be permanently sealed with a ring of gum, which will prevent the access of air or displacement of the cover in after handling. This finishing ring is put on in the same manner described for making

the cell-wall; and the precaution is taken to spin first an aqueous cement to prevent the solvent of the gum-cement from running under the edges of the cover and staining the balsam, because it must be remembered that the same solvent will dissolve both. In spinning the final ring, bring the brush in contact with the circle and slip at the same time, so that the ring will lap on each, and thus make a perfect sealing. The slide is now properly labeled and placed aside to dry. If the damar cement is preferred, the steps are the same as those given in the case of balsam.

If the sections are too thick, however, to take the cover-glass without a ring to raise the cover above the slip, the slip should be prepared with a ring some time before the sections are placed in position, in order to give the gum composing it time to thoroughly harden. This primary ring can be as high as desired by repeated application after each coating has had time to first dry and harden. In making the mount, the edges of the cover-glass should reach only as far as to the centre of the ring, and it will thus be possible to seal the mount with the last ring already spoken of.

These gummed rings are made of either Brunswick black, shellac, King's cement, asphalt or other like solutions dissolved in xylol, benzole, etc. The spinning is accomplished by means of a small camel's hair brush, held with a steady hand in contact with the glass slip, while the table is rapidly whirled by the other hand. The brush should be so held that a line drawn from the person through the centre of the table will make nearly a right angle with a line passing through the length of the brush handle. The cell wall should be $\frac{1}{8}$ of an inch wide, and should project about half that space beyond the edge of the cover-glass.

"When aqueous cements are used, they must be covered, after they have set and dried thoroughly, with a resinous cement that will be impervious to, and unaffected by, the

atmosphere. An analogous operation enables us to protect a resinous cell-wall from being attacked by similar mounting media. In such cases all that is necessary is to cover the wall with an aqueous cement, which, when dry, acts as a complete protection to it. This enables us to use one and the same cement for the foundation for all cells, whether containers for balsam or glycerin, damar or gelatin, leaving subsequent manipulations to be determined by the mounting medium to be employed. The importance of this apparently trivial point is that it enables the microscopist to keep on hand, at all times, a stock of thoroughly seasoned cells ready to be filled when wanted."

Glycerin Jelly Mounting.—The fixing of the sections to the slip and staining are in all respects the same for mounting in glycerin as already described. In the case of balsam, the slip and cover-glass are placed in the water bath, but before doing so, however, a small lump of the glycerin jelly is transferred to the centre of the cover-glass. As soon as the glycerin melts, the slip is centered on the turn-table and the cover-glass put in place with the forceps the usual way (see page 96). Care must be taken that the heating in the water bath is not greatly prolonged, because there will be danger of introducing many air bubbles in the glycerin. If air bubbles should appear, they must be exploded before the cover-glass is put on, and a close examination must be made for them by means of the hand magnifying glass. By placing the jelly on the cover-glass instead of the slip, the chances for air bubbles are greatly reduced. With the spring clip in position on the slide, the surplus glycerin is carefully washed off and the surface of the glass thoroughly dried by means of blotters. A ring of the glycerin jelly is now spun around the edges of cover-glass and the slide is put aside under a bell-glass to season and harden, after which the finishing gum is spun over the jelly and the slide is then labeled. It must be borne in mind that the spinning of a ring of glycerin jelly around

the edges of any of the mounts containing Canada balsam or other reagent will greatly prevent the running under the cover-glass of the gum comprising the finishing ring, which is so apt to occur unless the protection of the jelly is interposed. The jelly, in each case, must be allowed to congeal before the gum is spun on. The student is referred to the chapter on receipts for a suitable cement for finishing his slide. King's, Bell's or Lovett's cements will be excellent.

Dry Mounts.—It is only necessary to use mounting media in those cases where preservation is demanded, but in some instances the specimen may be mounted without the media, particularly when the slide is intended only for temporary use, and there is no desire to permanently preserve the form. Under these conditions the specimen is attached to the slip by means of some transparent cement—Canada balsam will do very well—and a *thin* ring of cement is spun around the well seasoned cell and the cover-glass is firmly pressed in contact with it, and the slide is then placed aside to dry, after which the finishing ring is applied if desired.

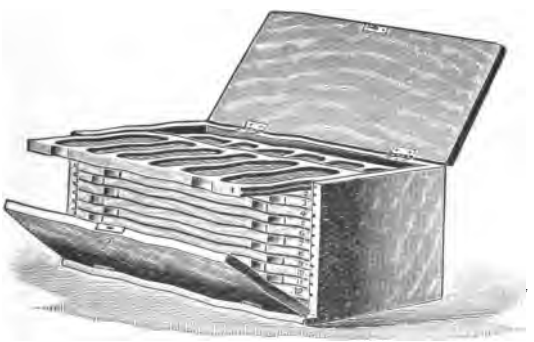


Figure 44. Case for Microscope Slides.

CHAPTER VIII.

How to Draw the Sections and Make Photo-Micrographs.

Before photography became so widely used—anterior to the introduction of dry-plates, and the many facilities for rapid and comparatively easy working—drawing was the only method available for readily transferring to paper what was revealed in the microscope. Even now some microscopists of distinguished abilities prefer drawing to photographing the image. It is the opinion of the author that both methods should be used; drawing in some cases will be found best, while in others photographing will give the most satisfaction. Drawing must be entirely free-hand, and, of course, where the results are largely due to the judgment of the observer, the reproduction cannot in all particulars be as accurate as the exact impressions made on the sensitive plate by the photographic methods. But in order to secure the best results with the photo-micrographic apparatus, the sections must be very thin, so that all parts will be in focus when the picture is taken, otherwise an unpleasant blur will be produced. With some kinds of objects it is impossible to avoid the presence of several planes, and the readjustment of the microscope becomes necessary in order to bring out each part of the object clearly. Such specimens can only be reproduced by the camera lucida. The operator must be constantly raising and lowering the objective while drawing the details; such manipulation, of course, would be out of the question while using the photo-micrographic camera. The student must, therefore, learn to use both the camera lucida and the photo-micrographic outfit.

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The necessary apparatus required for drawing the image consists of a compound microscope, a camera lucida, stage and eyepiece micrometers, liquid India ink, sharp pointed steel pens and good lead pencils. Bernhard's drawing desk may be also added as a convenient aid, though not absolutely necessary.

The Camera Lucida.—This instrument is attached to the ocular end of the microscope and is used for directing the rays, which emanate from the object, in such a manner as to cause the virtual image to appear projected on the table at the foot of the microscope, where it may be

Figure 45. Abbe Camera Lucida.

drawn with a pencil. The fields of view on the drawing board, and on the stage of the microscope, are both combined in one eye, and the image on the drawing board is made visible by double reflection. One of the best camera lucidas in the market is made by the Zeiss Optical Co., of Germany. It is shown in the cuts (figures 45 and 46). "The drawing surface is made visible by successive reflec-

tions at a large plane mirror and the silvered surface of a small prism in the eye-point of the eye-piece. The microscopic image is seen directly through an aperture in the silvering of the prism, to which is cemented another prism,

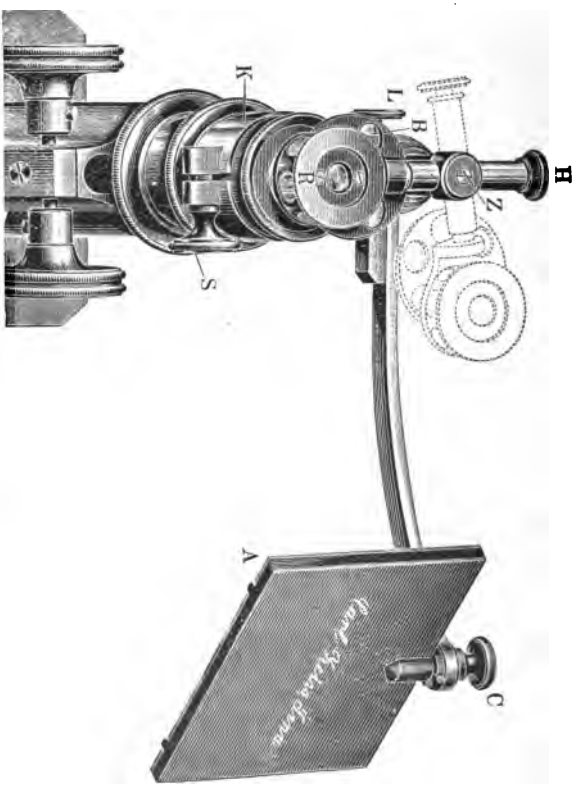


Figure 46. Abbe's Drawing Camera.

both prisms together forming a cube. Thus the pencils of light reach the eye coincidentally from both the microscope and the paper, and the image and pencil are seen without straining the eyes." [1] The rays from the drawing

1 Zeiss' "Microscopes and Microscopical Accessories." Page 82.

surface first pass through two tinted glasses of different degrees of color, which serve to equalize the illumination of the field and paper, and they will be found necessary when high powers are used for drawing. The arm upon which the mirror is poised is 10.5 cm (4 inches) in length, and it is not required to raise the surface of the drawing board unless in the case of very large drawings. This lengthening of the mirror arm greatly lessens the tendency to distortion. If the drawing is large, it will be necessary to raise or incline the surface containing the paper. The camera lucida illustrated in figure 46 shows a material improvement by Zeiss, in substituting for the tinted glasses a movable cylindrical cap, *R* in the wall of which is placed a series of tinted glasses, and by turning the cap on its upper edge until a small pin engages in a corresponding small hole on the lower edge of the cylinder, each smoked glass may, in turn, be brought in the path of the rays from the drawing surface. In the cylinder are five tinted glasses of different strengths, while the sixth hole is left empty.

For diminishing the brightness of the image a disc *B*, as in Bernhard's apparatus, with four smoked glasses and a vacant space, is interposed between the prism and the eye-piece.[1] In order to pass conveniently from observing the image projected on the drawing board to observation directly through the ocular, the instrument is so constructed that the prism and the diaphram may be turned on the pivot *Z* to one side and the microscope can be used as though the camera is not clamped to the tube. The eye-circle is accurately centered with the aperture in the prism by means of the two screws *H* and *L*. When this is completed, there should be no obstruction to the vision through the ocular field. Provision is also made in the instrument for a spectacle-glass, in order to render the drawing surface more distinctly visible. This fits in the recess in the top of the cap *R*. It is recommended by the

1 Ibid.

manufacturers that in order "to obtain a fairly large field, drawings free from distortions, a slanting or raised drawing

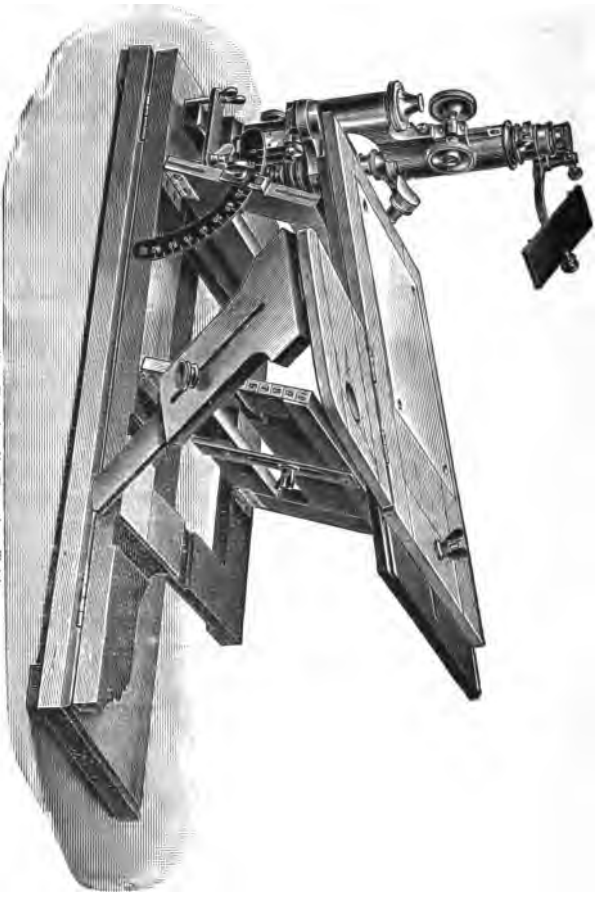


Figure 47. Bernhard's Drawing Table.

surface should be used."[1] The drawing desk devised by Dr. W. Bernhard is most admirable for this purpose. The

1 Ibid.

following points in its construction make it particularly well adapted for its purpose: The drawing board has a very exactly worked brass groove on the upper plate, so that any shifting of the parts is prevented. The height and inclination of the board are controlled by means of a graduated arc. There is an adjustable hand rest provided, and it is hinged to the drawing plate. A supporting piece is hinged beneath this hand rest which is capable of extension. The entire apparatus can be inclined from the plane of the table upon which it is resting, by means of hinge connections with a solid base-plate. This is shown in the cut.[1] "The plane of the drawing must be at the normal distance of distinct vision, *i. e.*, 250 mm. from the eye of the observer, since in general the drawing should correspond in its dimensions with the microscopic magnification, and therefore also the drawing desk must be adjustable in height and inclination to the microscope."[2]

In drawing it is generally customary to mark off only the outlines of the image on the paper, and the details are afterwards put in without the aid of the camera lucida. The size of the drawing is determined by the aid of a micrometer located in the ocular. Before beginning the drawings the ocular micrometer must be carefully compared with a stage micrometer, in order to determine its exact value, and it will be advisable to construct a table of known standard measures for each of the objectives, and then the stage micrometer may be placed aside and the ocular micrometer used instead. When the paper is put in position on the drawing board for reproducing the image, and the camera lucida has been adjusted in its place, the measurements should be marked on the margin of the drawing to show the degree of magnification of the image. When the work is finished in ink, this measurement should also be permanently marked on the paper. In work of a high

1 Journal of the Royal Microscopical Society, 1895. Page 102.

2 Zeitscher. f. wissensch. Mikroskopie, Vol. 9, page 439, 1892, and Vol. 11, page 298, 1894. Also Journal of Royal Microscopical Society, page 782, 1893.

grade, this indication of the magnification is absolutely necessary, and should always be shown on the drawings. The simplest way of securing this scale of enlargement is to first place the stage micrometer in its position on the stage, and draw through the camera lucida the lines of the measurement on the corner of the drawing; and "the enlargement of the figure can be determined at any time by measuring with a steel scale the length of the image of the micrometer spaces and dividing it by their known width. Thus, suppose the five spaces of the scale of enlargement given with the drawing were found to measure 25 millimeters, and the spaces on the micrometer were $\frac{1}{100}$ millimeter, then the enlargement would be $25 \div \frac{5}{100} = 500$. That is, the image was drawn at a magnification of 500 diameters." [1]

In drawing the object thus indicated, great advantage accrues to the student in making him careful about details and accurate in regard to results. He will find that a poor mount cannot be well drawn and he will, therefore, be careful from the start, to make all his work the very best possible. In doing this drawing the following items may be kept well in mind:

1. Use hard pencils, with fine, sharp points.

2. The drawing can be best made on Bristol board, or similar smooth-surfaced paper.

3. The light which comes through the tube of the microscope from the object, and also those rays which come from the image and are reflected by the mirror of the camera lucida to the eye of the observer, should be of nearly the same intensity, in order to bring out clearly both the point of the pencil and the image. There are several ways of regulating the intensity of the light at each point. If the stage is too bright, the Abbe sub-stage illuminator will readily correct the trouble. If the microscope is not provided with the Abbe illuminator, then the dia-

1 "The Microscope and Mic. Methods," by S. H. Gage. Page 52.

phragm must be used with a smaller opening, or a screen must be placed between the stage and the source of light. To know which field is the brightest, the student must watch the image of the pencil and of the object, and if the latter is the most distinct, then he will at once know that the stage of the microscope is too bright; if the point of the pencil is the most distinct, then the surface of the paper has too much light on it. The character of light coming from the surface of the paper can be controlled by the camera lucida, if Zeiss' pattern is used, as has already been indicated. This, however, may be accomplished also by placing a screen of ground glass between the source of light and the drawing board. These mechanical devices, however, for controlling the quantity of light can be multiplied by the ingenuity of the student.

4. Fasten the paper securely to the drawing board so that it will not be displaced during the operations.

5. Follow faithfully the lines of the image, taking care not to make heavy markings with the pencil. Make all cell-walls, of an appreciable thickness, with double lines, and the solid portions of the sections should be well shaded.

6. Note on all drawings not only the magnification, but also as well the number or letter of the objective and ocular. This is not often done on drawings, but it is very desirable when work of a high grade is performed, in order that satisfactory comparisons may be made at any time.

7. If the outlines of the image are made with a low power, and the details are put in with a higher power, this fact must be clearly indicated on the drawing.

8. The eye must be held close to the microscope, and look perpendicular down, so as not to distort the image and produce an incorrect drawing.

9. The height of the drawing desk must be lowered or elevated until its surface is brought into the distinct visual distance of the observer. This height can be determined by the distinctness of the inclination of the desk by taking

a stage micrometer and draw its measurements on the paper; if the lines of the divisions stand at unequal distances, separating more and more as you approach the top of the desk, the surface needs inclining.

Photo-Micrographs.—The remarkable strides made in the science of photography within the past few years have rendered it possible to reproduce with great exactness the image of the object, and with such a degree of facility as to place the results within the reach of any microscopist who possesses a general knowledge of chemical laws, and who will exercise the ordinary degree of care in the use of delicate apparatus and chemicals.

Before entering upon a discussion of the methods used for making the negatives and positives, it may be best to give a detailed account of the apparatus and chemicals required for a satisfactory prosecution of the work. In the descriptions which follows it is assumed that the student is a novice in the science of photography, and therefore, the subject is treated fully in order to make the methods clearer to the minds of the beginners.

As in the case of the microscope and its accessories, so is it true in reference to the photographic outfit: the best apparatus made by the most skillful workmen, will be the cheapest in the end. A lens made to sell and offered below what is a reasonable price for a first rate glass will soon be found inadequate to meet the demands, and only inferior pictures will be the results.

I. The Camera.—The following sizes of view cameras will be found very convenient and useful in the laboratory: 4 x 5; 6½ x 8½. The different sizes of kits should be purchased with the cameras, so that the several sizes of negatives may be made without requiring the purchase of other cameras. It will be possible to do all needed work with the cameras above mentioned, but in a well equipped laboratory it will be found desirable for con-

venience to add also an Eastman's No. 4 Kodak and Bausch & Lomb's Professional Photo-micrographical camera, or Zeiss' large Photo-micrographic and Projection apparatus. These two last are very expensive, but the laboratory that contains one of them is prepared to do work of a high grade with the least degree of inconvenience. Figure 48 is a camera very well suited for ordinary purposes, It is called

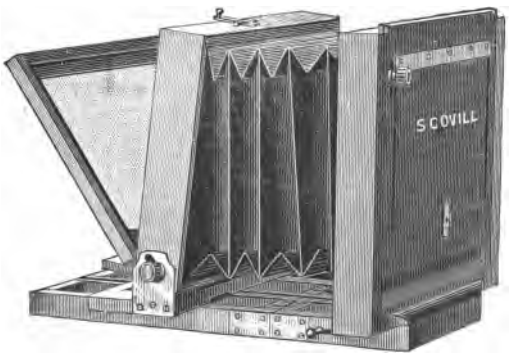


Figure 48. Waterbury Camera.

"the Waterbury" camera, and is well and strongly made, with a very few "extras" attached. The finish is not equal to the more expensive apparatus, but the parts are accurately made and the fittings are all true. The 4 x 5 camera may be of this pattern, and it will do the work required of it as well as a more costly instrument.

Figure 49 is an illustration of a camera made by the Eastman Company, of Rochester, N. Y., and is one of the most beautifully finished and well adapted instruments to be found in the market. There are several properties belonging to this camera which make it peculiarly valuable for general work in the laboratory. It has, for instance, a double rising front for lowering or elevating the lens with-

out disturbing the position of the stand. This gives wide range of adjustment. It has also a double swing, which enables the operator to secure a clear cut and distinct focus on the object. The ground glass plate is kept stationary while the focusing is being accomplished, and the front of

Figure 49, Eastman's Interchangeable View Camera.

the camera is moved by a rack and pinion adjustment. The advantage in this method over the usual way of focusing by moving the back of the camera, can be fully appreciated by the photographer. The reversible back holds the ground glass plate in such a manner as to permit of it being lowered by means of hinges, without entirely sepa-

rating it from the camera, thus avoiding the danger of breaking while introducing the plate-holder. Figures 50, 51 and 52, show the different positions the frame containing the ground glass may be placed in while manipulating the camera. Figure 05 shows the frame closed, ready for focusing; 51 open for receiving the double plate holder; and 52 the ground glass turned down, in order to permit the roll holder to slide into

Figure 50. Ground Glass Frame Closed for Focusing.

Figure 51. Ground Glass Frame Open, for Receiving Double Plate Holder.

Figure 52. Ground Glass Frame Open for Receiving Roll Holder.

position. Figure 53 shows the camera with the roll holder attached and ready for the exposure.

Figure 54 represents the Eastman's Folding Kodak

camera, which is constructed for receiving the roll holder and also glass plates. It may be used as a hand camera, or mounted on a tripod at pleasure. It has attached to it a

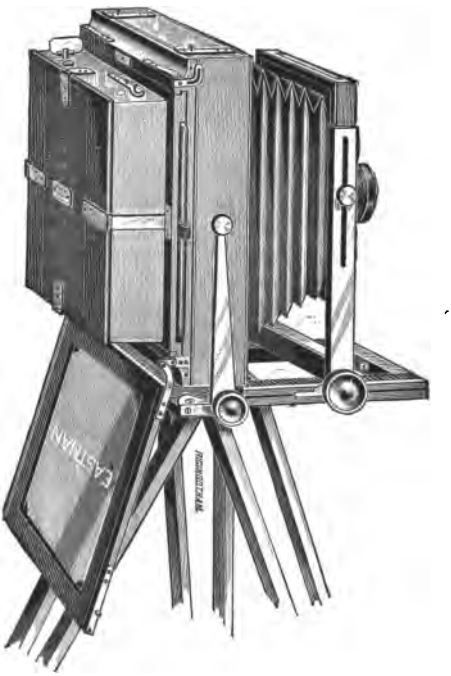


Figure 53 Eastman's Interchangeable Camera, with Roll Holder.

view finder, so that the operator may take instantaneous pictures; and at the rear there is a ground glass plate so that focusing can be accomplished in case the instrument is used for time exposures on the tripod and glass plates are desired. The camera, when not in use, can be folded up in the leather case shown in the cut and put in a very compact form, which is quite a desideratum in field work.

Much time can be saved in the making of the negative by the use of the roll-holder, which is constructed to con-

tain a roll of thin celluloid highly sensitized and so mounted in the holder as to permit of being brought, by the mechanism of the holder, a portion at the time, before the lens, and thus receive successively on its surface as many as 48 or

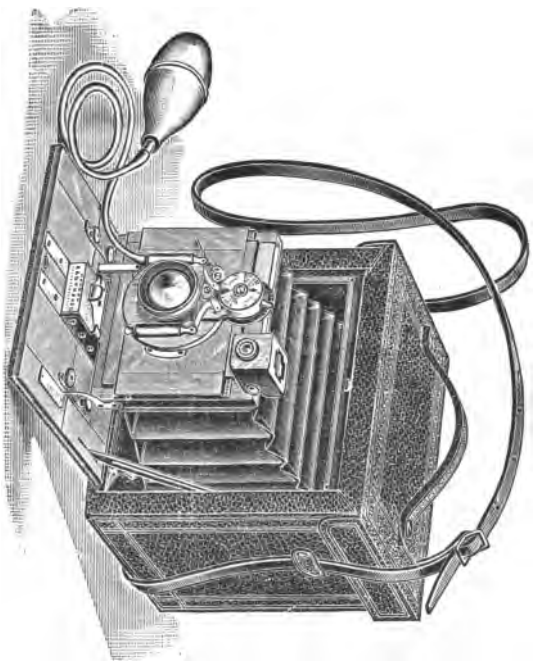


Figure 54. Folding Kodak.

even 100 pictures before requiring the operator to return to the dark room and reload. Figure 55 shows the roll-holder closed ready to be attached to the camera, and figure 56 is a view of the roll of sensitized film before it is transferred to the holder.

In order to place the roll of film in the holder, the following instructions should be carefully observed: "Place the holder face down on a level support, and by pressing down and in upon the hinge clasps (fig. 57), remove the

downward pressure from the hinges, at the same time pressing the lower ends in and they will spring out from under the plates with which they are engaged." In the case of the roll-holder used with the folding kodak, there is a slightly different fastening, rather more simple in its application, and more readily unclasped.

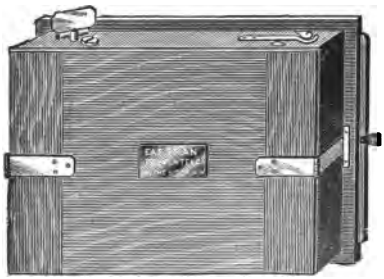
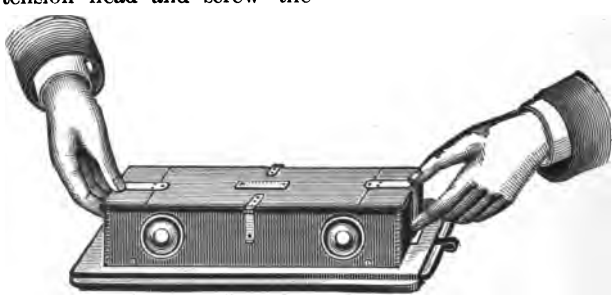


Figure 55. Eastman's Roll-Holder. Model of 1889.

"Remove the back and lift the body of the roll-holder out of the shutter frame, as shown in figure 58." This is not required with the kodak holder, it is rather more simple in its construction. Place in "the spool by fitting the slotted end upon the wing of the tension head and screw the

Figure 56. Spool of Film Secured with Paper Band.



Figuge 57. In the Act of Opening the Roll-Holder.

thumb screw back in its place (figure 58). Take hold of the end of the paper and pull it out about one and a half times the length of the exposure, and thread it through between the

end of the case and the measuring roll (see figure 60). With a pair of scissors clip off the corners of the strip so it will go under the reel clamp. Thread the end of the paper over the second guide roll, and after lifting the clamp of the reel, thread it down under it and pull it flat and even (figure 61), making sure that it lies flat and straight on the exposing board in front. Then press the clamp down tight. Turn the measuring roll forward until the click spring is just ready to snap, and then tighten the paper by giving the reel a partial turn backward until the spring snaps down off the indicator. Make sure that the pawl is on the ratchet of the reel, so that it cannot turn backward and loosen the paper (see figure 62). Set the holder box into place in the shutter frame and lay on the back and press the clasps into place as in figure 63. In putting the holder together, see that the slide is in position to draw to the right, that the key and indicator are on the farthest side, and that the name plate on the back faces the operator, as in figure 55." [1]

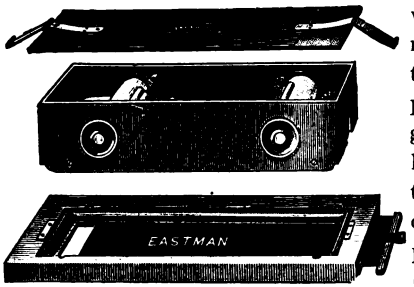


Figure 58.

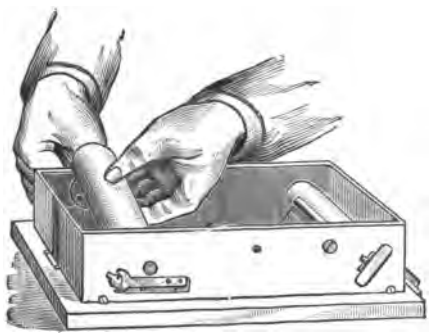


Figure 59.

1 Eastman' instructions for using the Improved Roll-holder

After the exposures have been made and it is desired to cut the film into the separate pictures, open the holder as

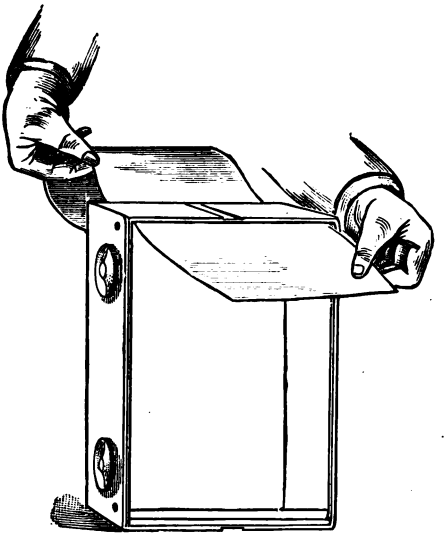


Figure 60.

already described, and throw off the pawl from the wheel on which it bears and pull out the film after it has been cut with a pen knife at the end of the last exposure. In pulling the film out it will

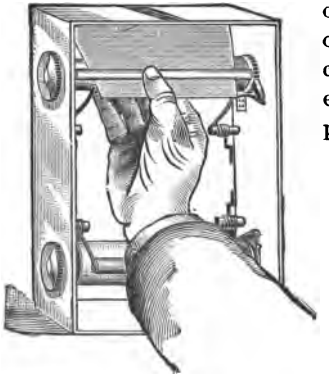


Figure 61.

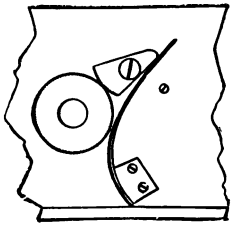


Figure 62.

be noted that on each side there are a series of holes punctured in the film. These perforations have been made by the measuring roll and indicate the positions at which to

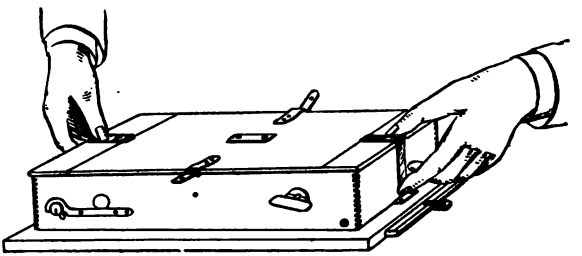


Figure 63.

cut in order to separate the pictures from each other. Figure 64 brings out this fact clearly.

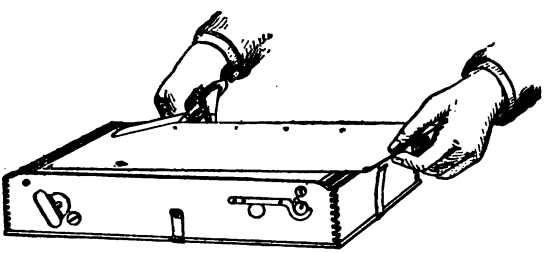


Figure 64.

The Lens.—Two sizes of the photographic lens will be found sufficient for the usual work in the laboratory—4x5 and 6½x8½. A wide angle lens will also be found very useful in certain kinds of work, and it will be well to also purchase one of these for the laboratory. The following lenses are considered to be among the best now offered in the market:

Alvan G. Clark Lens.
Beck's Autograph.
Dallmeyer's Lens.
Darlot's Lens.
Francais's Extra Rapid Rectilinear.
Gundlach's Lens.

Morrison's Lens.
Queen's Pantagraph.
Ross' Lens.
Steinheil's Lens.
Suter's Aplanatic.
Voiglander Lens.
Zeiss' Anastigmat.

The above list may be classified as follows, according to merit:

1. Zeiss' Anastigmats.
2. Dallmeyer.
3. Beck's Autograph.
   Suter's Aplanatic.
4. Alvan G. Clark's Lens.
   Gundlach.
   Voitlander.
   Steinheil Aplanatic.
5. The others mentioned in the list may be placed under this head.

In the selection of a photographic lens, the following requisites must be carefully noted:

1. Achromatism, of course, is of first consideration, and the lens must stand the test well, because a slight deviation from achromatism will produce an indistinct image.

2. Distortion of the image on the outer limits must be reduced to the least degree possible.

3. Rapidity in action and rectilinear in results are absolutely required in all first-class lenses. The rapidity of a lens depends upon the size of the stops in the diaphragm. The larger the opening more light can enter and quicker will be the action on the sensitive plate. The size of the diaphragm is controlled by the focal length of the lens; that is to say, if aperture f 16 be used, it signifies that size of the beam of light which enters the lens is one-sixteenth of the focal length. This property of the objective has been carefully worked out by the Photographic Society of Great Britain, as follows:

Aperture: f4–f5.6–f8–f11.3–f16–f22.6–f32–f45.3–f64.
Relative exposure ratio: 1–2–4–8–16–32–64–128–256.
By the adoption of this series of apertures one lens

can be compared with another. In the use of the stops it must be remembered that each requires twice the exposure of the one preceding to produce the same results on the sensitive plate. The special advantages in their use, however, consists in reducing to a minimum the blurring of the image on the outer margins, and in producing sharpness in detail which is especially pleasing in the picture. The small stops also increase the depths of definition.

4. The angle of view should not be less than 50° for the rectilinear type, while the wide angle series should cover an angle of not less than 85°.

5. Depth of focus depends on the angle of view, working aperture and focal length. As these increase the depth of the focus decreases, and vice versa. Depth of focus is due to the converging rays striking the plate at a more acute angle.

6. Flatness of image over the entire surface of the ground glass-plate by extending the oblique pencils of light.

7. Depth of definition to give a sharp, clearly defined image on any portion of the plate, by reducing spherical aberration. When the focus of the lens is shortened the greater will be the depth of definition. Thus it is that in the case of a short focus lens there will be projected on the sensitive plate a greater number of well defined objects in the foreground than will be secured in the use of a lens of longer focus. This is a valuable property in landscape work where distance comes in as an important factor.

Camera Stand.—This should be so constructed as to permit of raising and lowering the top on which the camera is placed, in order to bring the principal axis of the lens in coincidence with the centre of the object to be photographed.

THE FOLLOWING ADDITIONAL APPARATUS will be required for making the negatives and positives:

Focusing cloth and focusing glass.

Double plate holders.

Eastman roll-holder.

Diaphragm shutter.

Ruby lamp for the dark room, either in the form of an electric lamp or gas or oil.

Developing trays of various sizes.

Graduates for measuring the chemicals.

Negative washing box.

Negative drying racks.

Light-tight boxes for holding the different sizes of sensitive plates.

Kits of various sizes for use in the plate holders.

Hard rubber fixing baths, various sizes.

Printing frames.

Retouching frame.

Camel's hair brush, two inches in diameter for dusting the plates before exposing them in the camera.

Burnisher.

An enlarging easel.

Eelectric retouching pencil or air-brush.

Color screen to use with the orthochromatic plates.

Scales for weighing out the chemicals for all of the photographic operations.

Glassware such as beakers, flasks, etc.

The necessary chemicals used in the photographic manipulations will be considered when the formulæ are given in describing the methods for making the negatives and positives. But it must be borne in mind that only the purest chemicals should be purchased and used in developing the negatives and making the lantern slides. Inferior results will meet the operator unless he observes rigidly this rule.

CHAPTER IX.

MAKING THE PHOTO-MICROGRAPHS, CONTINUED—THE DESCRIPTION OF THE APPARATUS AND THE SOURCE OF LIGHT.

Arranging and Adjusting the Apparatus.—Some place in the laboratory must be found where there is the least degree of vibration, caused by passing to and fro in the building, and from outside influences, and there the table containing the appartus for focusing and exposing the sensitive plate must be located. If the building has a well lighted and ventilated basement, with a cemented floor, no better place can be secured for performing all the work in photo-micrography. It must be remembered, however, that wherever the apparatus is located decided vibrations given to the table will sadly spoil all results by producing a blurred picture. All parts of the apparatus used for holding the slide, the camera and focusing appliances and the condensers for concentrating and controlling the distribution of the light, should all be fastened to the same rigid base, so that if slight vibrations occur in the room the entire outfit will move as a whole, and there will be less tendency to blurring the negative.

Since the temperature of the atmosphere influences, to a very large degree, the results secured in photographing, it will be of great advantage to have the room under perfect control, so that the temperature may be lowered and raised at pleasure. It has been found, from experience, that the best negatives are made when the temperature of the chemicals range from 60° to 70° F. If these solutions used in developing are not kept within this range, the negatives will be unsatisfactory. Cold retards the results, while heat accelerates and causes flatness. In summer, therefore, ice

should, be kept around the vessels containing the developing solutions until the temperature is lowered to the required degree, and the dark-room must also be regulated, as far as possible, to reach these same results.

Although there are several excellent brands of dry-plates on the market, all giving good results in the hands of the careful manipulator—still, it will be wise to study the qualities of the plates made by one manufacturer and use them in most, if not all, experiments conducted in the laboratory. In determining this question, careful notes should be taken of each exposure, and also the action of the developing fluids on the plates. By this method all of the capabilities of the plates will be fully understood. The experience of the author has convinced him that, for general scientific work, Carbutt's plates are superior to most of the brands sold in America. This conclusion, however, may be due to the fact that he is more familiar with these plates than with the others; but, as has been already stated, the same amount of experience with the others may yield as good results. Mr. Carbutt's plates give brilliant negatives and they allow considerable latitude in exposure and development. The following represent the brands he manufactures which are suitable for the experiments in the laboratory:

"Eclipse, sensitometer 27.—Films and plates are extremely sensitive, and specially intended for quick studio exposures, concealed and detective cameras, instantaneous views, and magnesium flash-light photography.

"Keystone special, sensitometer 23 to 25.—For portraits, instantaneous views, out-door groups, etc. Blue label. The best plate for view work.

"B plates, sensitometer 16 to 20.—For landscape views and general photography.

"Orthochromatic plates, sensitometer 23 to 27.—Gives correct color values. The best plates for landscapes, interiors, and photo-micrography.

"'A' gelatino-albumen plates.—Giving results of great brilliancy and fine color. For lantern slides and window transparencies. The sensitive film is mounted either on glass plates or celluloid.

"The sensitometer number on each box of plates indicates their sensitiveness; the higher the number the greater the rapidity of the plate. For portraiture on the special and eclipse plates under the skylight, if an eclipse, sensitometer 27, required 2 seconds, a special sensitometer 27 would require $3\frac{1}{2}$ seconds, and a special sensitometer 24, $4\frac{2}{3}$ seconds." (Carbutt.)

The orthocromatic plates are the best for all photo-micrographic work because they give color values, so important in photographing stained sections. Without these plates it would be impossible to secure clear, sharp pictures of the colored sections because the ordinary dry plates have not the proper tone-values—some of the colors appearing too dense while others are too light. The plate is orthochromatised by immersing it in a solution of a dye belonging to the eosine group, in accordance with the theory of Professor Draper, who discovered that only those rays which are absorbed by the film on the plate produce chemical changes in the sensitive surface. In using these orthocromatic plates it is necessary to interpose between the lens and the surface of the plate a color-screen in order to intercept and equalize the chemical action of the different colored rays which are emitted from the section. The color of this screen is generally yellow, although it is claimed by some experimenters that a tinge of red, blue or green is sometimes preferred. In the use of these plates two facts must be borne in mind: the exposure must be prolonged beyond that period given to the ordinary plates, and the developing tray must be covered while developing so that the plates will not be fogged by the light sifting through the ruby-glass. In order to have the dark-room window perfectly safe it will be well to cover the ruby-glass with

two thicknesses of yellow paper or cloth specially prepared for this purpose.

The apparatus illustrated in figure 65 is a simple contrivance for throwing the image on the ground glass of the camera and photographing it. It will yield very fair results when properly managed by the amateur student. It consists of a microscope placed in a horizontal position, and to the ocular end of which is appended a camera, while beyond the stage is suitably adjusted the lamp and condenser. The microscope is connected with the camera by means of a piece of black velvet so that all light is excluded except that which enters through the objective. The distance between the ground glass and the focusing appliances on the microscope is not so great but that the operator may easily reach it and arrange and focus the image on the ground glass plate.

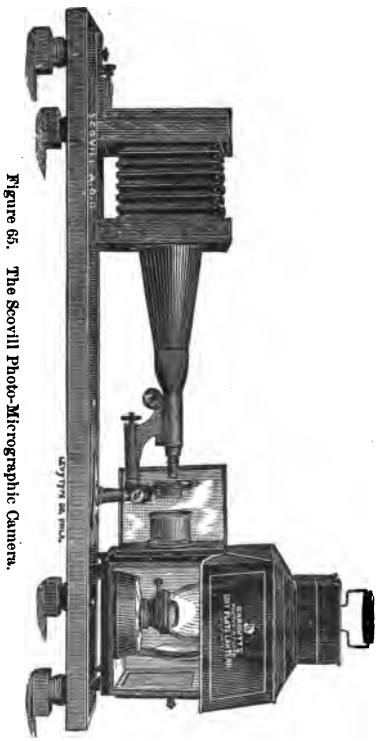


Figure 65. The Scovill Photo-Micrographic Camera.

Figure 66 is a photo-micrographic apparatus considerably more complicated and delicate in construction than the one just described. This apparatus is made by the Zeiss Optical Company, of Germany, and is costly; but the

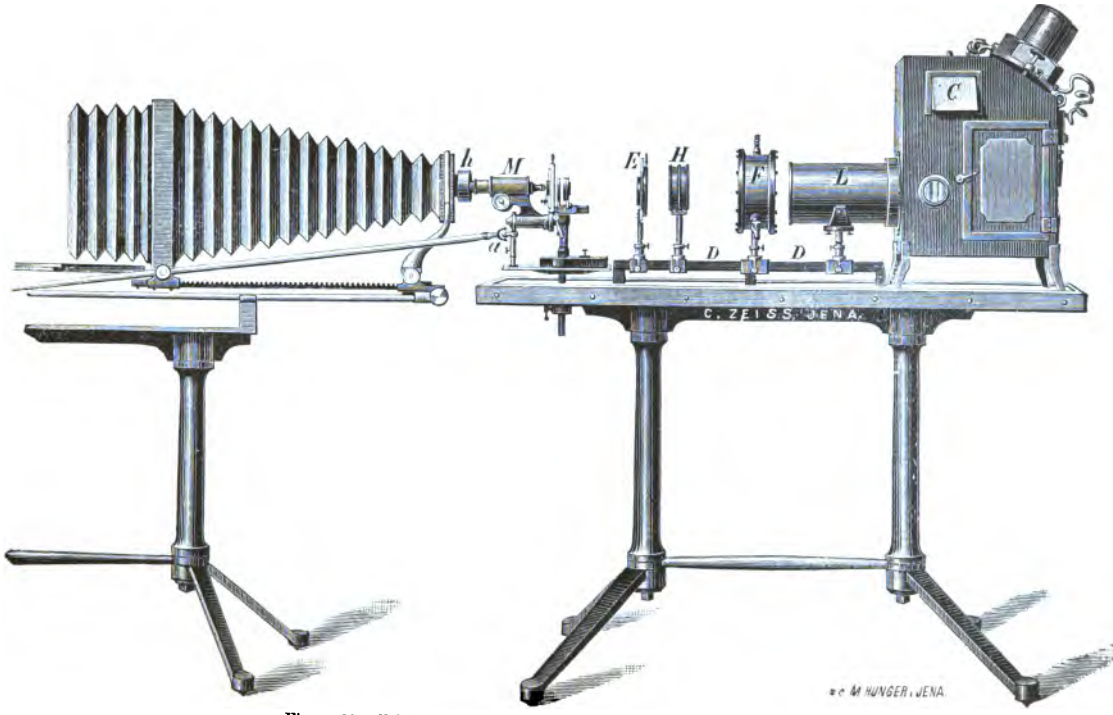


Figure 66. Zeiss' Large Photo-Micrographic and Projection Apparatus.

laboratory having such an outfit in its equipment will be fortunate. The appliance is not only used for making photo-micrographs, but it is also adapted for projecting directly on the screen the image of the object by removing the camera, which is attached to a separate mount. This feature of the apparatus is very valuable in certain kinds of experiments conducted in the botanical laboratory. The essential parts of the outfit are:

1. An adjustable sole-plate on which to rest the microscope.

2. A horizontal prismatic steel rail 100 cm. (40 inches) long, on which is arranged axially the parts of the apparatus for controlling the action of the light. This rail is called the "optical bench." (See DD in figure 66.)

3. Hooke's key for controlling the focusing screws (a).

4. Table screen, consisting of a black board suspended above the table, containing the projection outfit, with impervious curtains hanging from all the sides except the end over the microscope, so that all rays may be excluded from the instrument except those desired.

5. Absorbing cell for extracting the heat rays from the light radiated from the lamp, so that the delicate glasses of the lenses may not be injured nor the object destroyed. (See F.)

6. Iris diaphragm, having a total aperture of 6.7 cm. (2⅝ inches). This is intended to hold colored screens and cut off all light not desired in the illumination of the object.

7. "Collecting lenses, having a diameter of 12 cm. (4¾ inches) and consisting of two anterior lenses which, having their concave surface turned towards the light, and being placed so as to make the distance between the luminous centre and the edge of the lens amount to about 12 cm. (4¾ inches), render the rays nearly parallel without introducing excessive aberration; a single posterior lens, which again collects the parallel rays in a point situated about 40 cm. (16 inches) from the lens." (Zeiss.)

8. Lantern slide carrier, with micrometer movement.

9. Light filter stand (H), for holding absorbing liquids to be used while photographing stained preparations.

10. An electric lamp, with heliostat, or resistance coil.

11. Camera and plate holder.

12. Movable dark slide for determining the sensitiveness of the plate with the different powers of objectives. The plate is moved through the slide in such a way as to expose only a small strip at the time, so that it is necessary to use only one plate to determine the time ratios.

The list of accessories given for this photo-micro-camera is intended for use with the electric light; if any other source of light is used it will be necessary to slightly change the list.

The Source of Light.—With the great improvements developed in electricity within the past few years, the microscopist has but little room for complaint in regard to the lighting of his mounts for photographing. In those places, therefore, where electricity can be secured no other kind of illumination should be thought of in photo-micrography. But it is not in the power of every student to use this most excellent light and he is forced to look in other directions for illumination. Sunlight is, of course, preferable, but there are two difficulties in the way of its use. (1) Its intensity is a very uncertain quantity in doing delicate work, and it is exceedingly difficult to determine the exact time of exposure in each instance while the sun is gradually climbing the zenith and the rays are becoming constantly more and more powerful in their effects on the sensitive plate. (2) The sunlight is not always available, because of the occurrence of cloudy weather.

When it is possible to use the sunlight, and the operator prefers to do so, there must be attached to the apparatus a heliostat and porte lumiere, or similar contrivance for controlling the direction of the rays from the

sun and concentrating them at the right point for illuminating the object. The heliostat is an instrument with clock-work construction which causes the reflecting mirror to follow the sun, and thus project the rays into the tube of the microscope requiring but little if any adjustment of screws on the part of the manipulator.

Electricity.—Although this source of light is not as bright as direct sunlight, still the author considers it to be preferable for the reason that it is possible to have it under thorough control. Moreover the intensity is entirely sufficient for all purposes in the laboratory, and the use of sunlight for projection may be dispensed with.

Electricity is used in the production of two kinds of lights, the incandescent and the arc. The latter is the most intense, and for a long time it was not practicable to use it for projection, for the reason that the carbons were so irregularly consumed it was impossible to keep the arc of the flame in thesame position in the optic centre of the condensers. Not until within very recent time has this difficulty been overcome. To Messrs. J. B. Colt & Co. is much of the credit due for devising an arc lamp which seems to satisfy all the demands reasonably made of such a light. The *Scientific American*, in speaking of this most excellent invention, says:[1]

"The lamp is perfectly automatic in the control of the carbons, which are disposed at such an angle as to present the crater of the positive carbon to the condensing lenses of the projector. This feature is very desirable, for, when carbons are vertical, even if the negative carbon is advanced toward the condenser out of line of the positive, the light will proceed from the negative carbon as well as the positive, thus making two sources of light instead of one—a condition fatal to definition on high class work. If, however, the carbons are placed at an angle, as shown in the cut, the luminous spot on the negative carbon is obscured

1 Scientific American, January 20, 1894.

from the condenser, and the crater on the positive carbon is presented in a most favorable way. By an admirable system of mechanism in the lamp referred to, the point of the lower carbon is accurately maintained in a given position, and the upper carbon is gradually and regularly fed toward it in the exact proportion to its consumption. This mechanism is so simply and nicely adjusted that the lamp may be run for hours without a flicker, always maintaining the radiant in the optical center of the projector.

"The regulating device is contained in a metal case, only 2 inches thick by 3¼ inches wide and 4½ inches high, making it the most compact and easily adjusted lamp that has been brought to our notice.

"The negative carbon is automatically moved upwardly as it burns away. The positive carbon is fed down by the weight of the carbon holder and by a small spring-actuated train of gearing, which is held in check while the arc is of normal length, or released when the length of the arc becomes too great, by a shunt magnet contained by the casing. The action of the shunt magnet is controlled by a spring acting on its armature; an increase in the tension of the spring increases the length of the arc, while a reduction of the tension diminishes the length of the arc.

"The mechanism controlling the carbons is so constructed that the lamp may be used on from five to twenty amperes of current, by the mere inserting of carbons of suitable sizes. The lamp is perfectly insulated, so it may be freely handled.

"This lamp may be clamped on a vertical post, with a sliding base, admitting of universal adjustment; or it may be mounted otherwise, as may be desired. The utility of this lamp is by no means confined to optical projection, for, owing to its convenient size and the absolute steadiness of its light, it finds extensive use in photo-lithography and copying, micro-photography, for theatrical effects, and, in fact, all uses for which an intense artificial light is desired.

The intensity of this light admits of the use of the optical projector in a room that is only partially darkened, which greatly increases its utility and widens its scope."

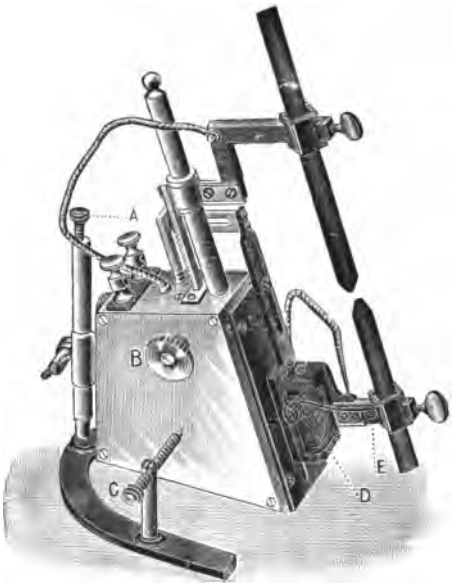


Figure 67. Colt's Automatic Electric Arc Lamp.

In figure 67, which is a representation of this lamp, *A* is a screw for raising or lowering the entire apparatus, so that proper centering of the light may be accomplished; *B* is a knob used in separating the carbons when the consumption requires the substitution of others; the magnets shown at *D* are for the purpose of separating the carbons when the current is turned on; *E* is the adjustment to enable the operator to place the lower pencil in the best position; *C* is a screw for adjusting the apparatus laterally, while on the opposite side is a spring to hold the instrument firmly against *C*.

As in the case of other electrical appliances, a rheostat is required in the use of this lamp, and the one illustrated in figure 68 is suitable for low tension currents. This rheostat is made by the same firm who manufacture the electric lamp shown in figure 67.

Figure 68.
Adjustable Rheostat.

Some suitable form of storage battery will be found convenient where a very steady light is demanded, although the lamp above illustrated will give a most constant flame from currents obtained directly from the dynamos. About twenty cells of the storage will be required to run the lamp. The current which comes either from the dynamo or the secondary battery must be direct or continuous and not alternating, and it should be from six to eight amperes and not less than sixty volts in pressure. A safety fuse should be placed in the circuit so that if any accident occurs, by short-circuiting, the installation will not be injured. If a short-circuit takes place while the wires are being attached to the lamp the safety fuse will be immediately burnt out before the instrument is affected. In as much as the electric motive force of the current usually received directly from the dynamos is 100 volts, it is necessary to introduce the rheostat or resistance coils (see figure 68) in order to change some of the current into heat, otherwise serious damage will be done to the apparatus. This rheostat also regulates or compensates any variation in the flow of the current of electricity.

Before the lamp is lighted the two carbons are in contact and as soon as the current begins to flow the ends of

the carbons become heated and the mechanism of the lamp separates them immediately and the arc is at once established. The theory explaining this action is that electricity, at the usual pressure, will not pass through a space of cold air greater than $\frac{1}{1000}$ of an inch unless filled with the particles of the incandescent carbon. When the separation takes place the arc is maintained by the rapid passage of the vapor or particles of the highly heated carbon. In connecting up the wires to the lamp it must be remembered that the carbon containing the crater must be placed in connection with the positive pole of the dynamo or battery and the carbon containing the point must be joined to the negative pole. The first or positive pencil must be at the top of the lamp, and both pencils must be inclined so that the light will not be projected downwards, as will be the case if the carbons are kept in a vertical position, but thrown out directly in front along the optic axis. There is a limit, however, to this inclination, because, since the lower pencil is slightly in front of the upper, too much inclination will cause the point of the lower carbon to be projected on the screen in the form of a shadow. The best length to be given to the arc for steady results is from $\frac{1}{8}$ to $\frac{3}{16}$ of an inch. When it is too short there is an unpleasant hissing and spluttering noise indicating an insufficiency of current, and in that condition the rheostat must be regulated so as to give more current to the lamp. In the lamp, however, illustrated in figure 67, there is a mechanism provided for controlling the distance between the carbons. The positive carbon is consumed faster than the negative, and it is at all times kept in the form of a crater from the depths of which come the luminous rays that are projected into the lantern.

Oxy-hydrogen or Lime Light.—This light is produced by the heating to incandescence a stick of lime under the combined action of burning oxygen and hydrogen gases. But little need be said, however, concerning this method of

lighting since the electric current is so readily secured in any well equipped laboratory, and there is so little inconvenience as well as danger connected with its use. The oxy-hydrogen illumination, unless managed with the greatest care, is so liable to give serious trouble and there is always an element of danger associated with it, because of the explosive properties of the gases, which make its use in the hands of students unsafe. If, however, such a light is desired the gases can be purchased compressed in metal cylinders ready for immediate use with but little cost. Or, these gases can be generated in the laboratory with but little trouble, provided the apparatus is secured. The oxygen is made by heating in a retort a mixture of one part of black oxide of manganese with four parts of chlorate of potash. Be careful to have the retort perfectly clean and thoroughly dry, and use only the purest chemicals. If they are pure they will melt on an iron spoon over a lamp without an explosion. Pass the gas from a retort through a purifying bottle and then into the metal cylinder. See that all connections are open and clear of all obstructions, and let the gas flow a short time before connection with the cylinder. All the air possible must be excluded from all parts of the apparatus to insure good results.

The hydrogen gas is generated by the action of sulphuric acid on metal zinc. Pass the gas through a purifying bottle as in the case of the oxygen, and the same care must be taken in its preparation as indicated in the manufacture of oxygen, in order to prevent explosions and serious damage to person and property.

The following apparatus will complete the outfit for producing the light:

Oxygen and hydrogen cylinders.

Pressure gauges for the cylinders to measure their contents. (See figure 69.)

Light regulator valves.

Oxy-hydrogen mixing jet for holding the lime pencil and mixing the gases before they strike the lime.

Hood for holding the lamp fixtures.

And, if the gases are made in the laboratory, generators, retorts, wash bottles and rubber tubing.

Gas Lamp.—The best form is Welsbach's gas burner

Figure 69. Pressure Gauge.

and reflector as devised by J. B. Colt & Co. This burner gives a strong light and will answer very well where electricity or the lime light is not available. In fact, it will serve most excellently in all cases where low power objectives are used.

CHAPTER X.

Making Photo-Micrographs, Continued—Focusing and Making the Negative.

The student should begin with the low powers first and not attempt too difficult work until he has mastered the simplest details. The arrangement of the apparatus must be carefully looked after and the parts all accurately adjusted so that all of the glasses will occupy positions in the same optic axis. There must not be the slightest leak of light in any portion of the outfit. It is best to use with the objective the projection ocular described on page 35, although good work has been done in the past by some of our leading microscopists by the use of the objective alone. Only the best glasses must be used in this work, because it will be found very difficult to make the actinic and visual foci coincide. With the use of the Jena apochromatic lenses, however, this difficulty is almost entirely overcome, and it is possible to secure objectives which are specially made for use with the photo-micrographic apparatus, with which when the visual image is focused on the ground glass an image equally sharp in its actinic effects is projected on the sensitive surface of the dry plate.

Mr. T. Comber, in the Journal of the Liverpool Microscopical Society, gives the following steps for adjusting the photo-micrographic apparatus with sunlight as the illuminant:

"1. Accurately centre the achromatic condenser, regulating the diaphragm so that the opening is a little smaller than the field.

"2. Remove the mirror from the heliostat, to ascertain that the spindle appears precisely on and in the field, which

it should do if the heliostat has been properly placed. Exactness in this adjustment is necessary, otherwise the beam of sunlight will not be motionless. The mirror is then replaced to reflect the sun in the centre of the field. At this stage the eye must be protected by a dark colored glass placed below the condenser.

"3. The object being placed on the stage is brought into the centre of the field and focused.

"4. The condenser is next focused to throw the sun's image exactly in the plane of the object. Sharpness of the ultimate image upon the ground glass cannot be secured without this.

"5. Changing the objective to one-sixth (4 mm.), I next measure the thickness of the cover-glass, or rather the distance between that plane of the object which it is desired to photograph and the upper surface of the cover-glass, by means of the fine adjustment screw. The purpose of this is two-fold: 1st. To facilitate cover-correction; 2nd. To ascertain whether the 2 mm. object glass, which is now put on, can get down to it, for its front lens is rather more than a hemisphere. * * * *

"6. The illuminating cone thrown by the condenser has to be regulated. The width of the cone should vary according to the nature of the object and the quality of the object-glass. Too narrow a cone produces diffraction fringes, that bane of photo-micrography; too wide a cone produces haze. To get true images, the cone, whether it be wide or narrow, must be absolutely axial. Even a very slight obliquity renders the images unreliable.

"7. The ordinary eye-piece is now changed for a projection eye-piece, set at the distance at which the sensitive plate is to stand; the camera is attached, and the long focusing rod coupled on (see figure 97). The image of the sun will be found in the centre of the ground glass. If it is not, the centering of the condenser must be wrong, and will require alteration. The sun's image should be sharp at the

edge, unless the sky is hazy. The image of the object, as seen against that of the sun, will be somewhat out of focus, but a slight turn of the focusing-rod brings it right." [1]

The instructions contained in the above copy from the article by Mr. Comber will also apply when the electric lamp is used in place of the sun. Proceed in the same manner in all respects while focusing, except that the electric lamp is substituted for the heliostat.

Since it is the common practice of microscopists to stain the sections, in order that distinctive contrast in the systems of cellular structure may be secured, it will be found difficult to produce good negatives, presenting clearness in detail, with the use of the ordinary dry plate. It has been found necessary, therefore, to submit the plates to a special treatment, so as to correct them for color values. The student can prepare the ordinary dry plates by immersing them in erythrosin and cyanin solutions. When so treated the plates are called *orthochromatic*, and they may be purchased from dealers sensitized for the red and green colors. A most excellent account of the method for preparing the plate is given in Anthony's Photographic Bulletin for 1893, page 608, extracts from which are given below. The article from which these items were gathered was read before the Societe Francaise de Photographie, on May 5th, by M. Monpillard. [2]

"*Orthochromatic sensitiveness.*—Though chemical orthochromatic plates are sensitive for the green and red, and generally give satisfaction, M. Monpillard says that, for scientific purposes, he prefers ready orthochromatized plates, which, when used shortly after preparation, have a maximum of sensitiveness to the luminous radiations. The operation of orthochromatization demands only elementary care. The dark-room lamp should have two thicknesses of deep ruby glass, the flame being reduced to as small a

1 See also Journal of Royal Microscopical Society.

2 See also Journal of Royal Microscopical Society for Feb, 1894, page 113.

degree as convenient during the bathing of the plates After the plates are bathed they are passed through three dishes of distilled water, and are finally dried in a drying cupboard containing a vessel in which calcium chloride is placed.

"For photomicrographic purposes the following colors give the best results: (1) Erythrosin (for green-yellow, yellow, and yellow-orange); (2) Cyanin (for red-orange and red).

"M. Monpillard says the following formulas have given him satisfaction:

"Erythrosin (stock solution): Erythrosin, 1 part; distilled water 1,000 parts.

"Sensitizing bath: Stock solution of erythrosin, 4 c cm.; water 100 c cm.; ammonia, 0.5 c cm.

"Cyanin (stock solution): Cyanin, 0.1 part; alcohol (95 per cent.), 100 parts. Only a small quantity of the solution should be prepared and it should be kept in the dark.

"Sensitizing bath: Stock solution of cyanin, 4 c cm.; water, 100 c cm.; alcohol (95 per cent.), 5 c cm.; ammonia, 1.5 c cm. The plates are immersed in either of the foregoing baths for two minutes, and are then washed and dried as directed.

"Erythrosin and cyanin plates bathed in both erythrosin and cyanin are rendered sensitive to yellow and red. The first bath consists of: Stock solution of erythrosin, 20 c cm.; distilled water, 80 c cm. After two minutes immersion, the plates are washed in two waters, and are then bathed in the cyanin solution given, washed and dried.

"Plates so treated are, it is pointed out, very much slower, but this is no disadvantage in photo-micrography, and on the other hand, they do not fog in development, which frequently happens when, to raise their general sensitiveness, the orthochromatizing bath is preceded by an alkalin bath.

"*Colored Screens.*—Colored screens may be used either in

the form of stained collodion, or, preferably, a small glass trough, with parallel faces, may be fitted with either of the following solutions:

"(1) For light yellow screen: Neutral chromate of potash, 1 grm.; water, 100 parts.

"(2) For deep yellow screen: Neutral chromate of potash, 5 grms.; water, 100 parts.

"(3) For orange screen: Bichromate of potash, 8 grms.; water, 100 parts.

"(4) For red screen: Erythrosin, 0.2 grm.; water, 100 parts.

"Number 1 weakens the blues and yellows; No. 2 extinguishes them; No. 3 cuts off the blue; No. 4 accentuates the action of the red.

"With those colored screens, and having sensitized the plates for given colors, it will be easy to obtain, in their true values, reproductions of objects, colored or uncolored. It is necessary, however, that the focus and the exposure should be made in the same monochromatic light, corresponding to a determined sprectrum color. This method of working assures the perfect sharpness of the image, inasmuch as the chemical focus is corrected.

"Where an object combines red and yellow colors, * * it would be better to sensitize for red and yellow and, according to the intensity of the former, expose with a deep yellow, or orange, screen. If blues and violets are found in the presence of yellows, oranges or reds, it would suffice to use a plate sensitized for the least actinic color (yellow or red), and as the plate is, of course, sensitive to the blues and violets, a yellow screen, pale or deep, could be used according as the more actinic parts of the object are more or less colored. For development, the author recommends hydroquinon with an alkalin carbonate and bromide, and the use of a feeble light in the dark-room."

The color screen can be placed in the front of the camera, in such a position that all the rays coming from the

object must pass through it before reaching the sensitive dry plate.

Exposures.—There are so many elements controlling the degree of exposure it is quite difficult to put down any fixed rules to guide the operator. The character of the light, the power of the objective used, and the condition of the object, are all to be well understood before this question can be determined, and these factors can only be known after careful experiment. As a general guide it may be stated that with orthochromatic plates used in connection with the color screen, the following figures give about the limits of exposures. These results have been determined by Mr. W. H. Walmsley, who has done some excellent work in photo-micrography. He used, however, a coal oil or petrolium lamp to supply the illumination, and in the substitution of the sunlight or electric light the time will be considerably reduced.

1½-inch objective, 3 to 45 seconds.
¾-inch objective, ¼ to 1½ minutes.
$\frac{4}{10}$ objective, ½ to 3 minutes.
⅛ objective, 2 to 7 minutes.
$\frac{1}{10}$ objective, 5 to 10 minutes.

The sensitive plate having been carefully dusted and placed in the plate holder—which, of course, must be done in the dark-room where no ray of white light can strike its surface—and the focusing of all parts of the apparatus having been thoroughly accomplished, as already explained, the exposure is made by attaching the plate holder to the camera in the place of the ground glass. The light is first excluded from the apparatus by placing an opaque card with a black surface between the objective lens and the stage, or, if very high powers are used, immediately beneath the stage. The slide is now withdrawn from the holder and, with watch in the left hand, the card is quickly raised, the light permitted to enter the instrument and project the image of the object on the sensitive surface of the

plate. After the exposure is completed quickly return the card to its place and then push in the slide. Now, unfasten the holder from the camera and return with it to the dark-room for the purpose of developing the picture.

Before opening the holder to take out the plate, prepare the following developing and fixing solutions. As a matter of illustration, we will take Carbutt's orthochromatic plates to show the method of treatment and his formulæ are given, therefore, in these instructions, but the student will find at the close of this volume other developers if he desires to use plates made by different manufacturers:

Eikonogen and Hydrochinon Developer.

Metric weight.	A	Avoidupois weight.
600 c. cm.	Distilled water	20 ounces
120 grammes.	Sulphite of soda crystals	4 "
22 "	Eikonogen	330 grains
10½ "	Hydrochinon	160 "
960 c. cm.	Water to *to make up to*	32 ounces

Metric weight.	B	Avoidupois weight.
600 c. cm.	Distilled water	20 ounces
60 grammes.	Carbonate of potash	2 "
60 "	Carbonate soda crystals	2 "
960 c. cm.	Water to *make up to*	32 ounces

	A		B		WATER	
For instantaneous exposures	30 c cm	1 oz	30 c cm	1 oz	120 c cm	4 oz
" Portraits	30 "	1 "	30 "	1 "	150 "	5 "
" Landscapes, Sen. 20–27	30 "	1 "	15 "	½ "	90 "	3 "
Full exposures, " 16–20						
" Lantern slides	30 "	1 "	25 "	¾ "	120 "	4 "
Full exposures	30 "	1 "	25 "	¾ "	120 "	4 "

and 2 to 6 drops restrainer D to each ounce of developer for lantern slides and full exposures.

NOTE—More of A will increase density, more of B will increase detail and softness. The temperature of the developer should not vary much below 65° nor above 75°.

Restrainer.

Metric weight.	D	Avoidupois weight
14 grammes.	Bromide potassa	½ ounce
150 ccm.	Water	5 ounces

Select a suitable tray for the size of the plate, take the plate from the holder and place it, sensitive side up, in the tray and pour over it *quickly* the developing solution. Now

gently rock the tray back and forth, so that fresh portions of the liquid will strike every part of the surface, as is shown in figure 74. If the plate has been properly exposed the image will begin to show within 15 to 30 seconds; but if it has been exposed too long to the action of the light, *overexposed*, as it is called, the image flashes up very soon after the developer strikes the surface and then rapidly sinks into the film and blackens all over the surface. If an *underexposure* has been made, the image fails to appear until after a minute or more has passed, and the details are flat and faint, the ground of the resulting negative being a sickly, grayish tint. In the case of a very much overexposed or underexposed plate, it is best to try again, and repeat the operation until the correct exposure is approximately attained.

Figure 74

After the details of the picture have come out well and the image begins to sink gradually into the film, the developing action is stopped by pouring the solution back into the graduate and washing the plate under the water tap. It must be remembered that the orthochromatic plate is very sensitive, and exposure for a time in any light which is safe for the ordinary plates will, after a short time, cause the negative to fog. The development must, therefore, be almost entirely accomplished with the tray covered. The plate is now placed in the fixing bath and permitted to remain under the action of the solution until all the soluble salts of silver are dissolved out and no white cloudiness is perceptible on the back of the plate. The following is the method of preparing the fixing solution:

Carbutt's Acid Fixing and Clearing Bath

4 c. cm	Sulphuric acid	1 drachm
480 grammes	Hyposulphite of soda	16 ounce
60 grammes	Sulphite of soda	2 ounces
30 grammes	Chrome alum	1 ounce
1920 c. cm	Warm water	64 ounces

During cold weather use only half the quantity of chrome alum given above.

"Dissolve the hyposulphite of soda in 48 ounces (1440 c.cm.) of water, the sulphite of soda in 6 ounces (180 c.cm.) of water, mix the sulpuric acid with 2 ounces (60 c. cm) of water, and pour slowly into the sulphite of soda solution, and add to the hyposulphite, then dissolve the chrome alum in 8 ounces (240 c. cm.) of water and add to the bulk of solution and the bath is ready. This bath will not discolor until after long usage, and both clears up the shadows of the negative and hardens the film at the same time."

When the negative has been fully treated with the fixing solution, it may be taken out into white light without any further danger of injury. Before drying it is thoroughly washed in running water for at least an half hour until all of the hyposulphite of soda is eliminated from the film. This washing is most conveniently done in the washing box illustratd in figure 75. The prolonged washing is necessary because the hypo salts hold on to the film with such tenacity, and, unless entirely extracted, the negative will soon become yellow and lose its brilliancy and value.

Figure 75. Negative Washing Box.

After complete washing, the plate is

swabbed with a wad of wet cotton and placed on edge in the drying rack to dry spontaneously. (See figure 76.) The drying will take place much sooner if the rack is situated in a draught of cool air. Care must be exercised, however, to prevent heat reaching the plate, but the drying must be in the shade and in cool air, otherwise the film will melt and the negative destroyed. When thoroughly dried the surface of the negative is coated with a varnish to protect it from the effects of the printing processes while making the positives. A permanent number is then made on one corner of the plate, and a corresponding number is also placed on the envelope or preserver, which contains the name of the object, date and such other data as may be desired for future reference. If the negative requires intensification, as is often the case with photo-micrographic work, the following method is recommended by Carbutt and is most excellent:

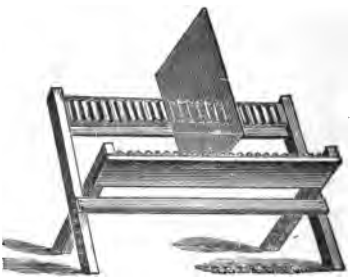


Figure 76. Negative Drying Rack.

Carbutt's Intensifying Solution.

No. 1.

16 grammes.....Bichloride of mercury.....240 grains
16 grammes.....Chloride of ammonia.....240 grains
600 c. cm .....Distilled water..... 20 ounces

No. 2.

16 grammes.....Chloride of ammonia.....240 grains
600 c. cm .....Water..... 20 ounces

No. 3. (Poison.)

180 c. cm .....Distilled water..... 6 ounces
4 grammes.....Cyanide of potassium C. P.....60 grains
60 c. cm.....Water..... 2 ounces
4 grammes.....Nitrate of silver.....60 grains

Mix the cyanide of potassium and the nitrate of silver

in separate vessels and pour the silver solution into the cyanide solution while stirring. "Flow sufficient of No. 1 over the negative to cover it, and allow to either partially or entirely whiten; *the longer it is allowed to act the more intense* will be the result; pour off into the sink, rinse, and flow over No. 2, and allow to act one minute; wash off and pour over or immerse in No. 3 until changed entirely to a dark brown or black. No. 3 can be returned to the bottle, but Nos. 1 and 2 had better be thrown away. Wash thoroughly and dry."

If the celluloid films are used for making the negatives instead of the glass plates, the method of procedure is the same, except that when the last washing has been accomplished the films are soaked for five minutes in the following solution in order to cause them to remain flat and not curl up:

750 c. cm.....Water.....25 ounces
30 c. cm.....Glycerin..... 1 ounce

Figure 77. Retouching Frame.

It often becomes necessary to touch up the negative in order to remove from it any defects that occur at times in the film, and this must be done before the final finishing varnish is applied. For retouching either graphite pencils or some opaque paint such as Gihon's is used. The best pencils for this purpose are L. & C. Hardtmuth's (Vienna) "black chalk points." Figure 77 shows a good retouching frame for holding the

negative and directing the light during the operation of penciling out the defects.

The Equipment of the Dark-Room.—Besides the items mentioned on page 12, the dark-room should be provided with developing pans, or dishes, or developing trays of the following sizes:

$3\frac{1}{2}$ x $4\frac{1}{2}$ inches. $4\frac{3}{8}$ x $5\frac{5}{8}$ inches.
$4\frac{1}{2}$ x $6\frac{3}{4}$ inches. $5\frac{1}{2}$ x $8\frac{1}{2}$ inches.
7 x 9 inches.

These trays must always be kept in convenient reach near the bottles containing the developing solutions and they should be used for no other purposes. The best trays are made out of hard rubber (figure 78) with two grooves running the length of the tray upon which the plate can rest, and thus furnish a convenient means of raising the negative when it is desired to examine the progress of the developing.

Figure 78. Hard Rubber Developing Tray.

On another shelf of the dark-room, sufficiently removed from the developing shelf or table so as to aviod all chance of mixing the chemicals, the hard rubber fixing baths to contain the hyposulphite of soda solutions may be located. (Figure 79.) The following sizes will be found suitable for most purposes:

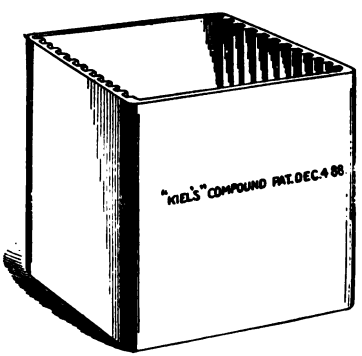


Figure 79. Hard Rubber Fixing Bath.

For $3\frac{1}{4}$ x $4\frac{1}{4}$ plates.

For 4 x 5 plates. For 5 x 7 plates.
For 5 x 8 plates. For 6½ x 8½ plates.

These fixing bath vessels are formed in such a manner as to hold the plates in a vertical position, and the grooves will permit one dozen negatives to be fixed at the same time. The sediment which may accumulate is kept from contact with the soft film by a ridge rising above the bottom of the vessel.

Several graduates or measuring glasses, of 4-ounce capacity, must also be in the dark-room for enabling the operator to secure the proper proportions of the developing agents. (Figure 80.)

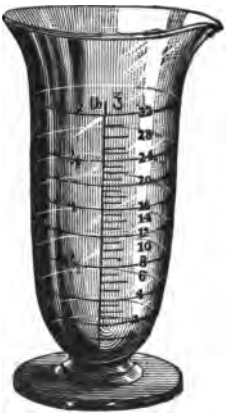


Figure 80. Graduate.

A minim glass will also be essential.

The light used in the dark-room must be carefully covered with ruby glass and also two thicknesses of yellow paper, or glass, to insure the proper protection of the plates while transferring them to the plate-holder

Figure 81. Carbutt's Multum in Parvo Lamp.

and developing. If electricity is not available a gas lamp may be used, or Carbutt's "Multum in Parvo" is an excellent lamp (figure 81.) This lamp has three doors; in one is an 8 x 10 inch plate of ruby glass; in the second is a plate of opal glass, covered with a metal door, which is used for examining the finished negative, and the third opening contains a clear glass through which the white light comes when the lamp is used for contact printing in making lantern slides.

CHAPTER XI.

Lantern Slides, How to Make Them, and the Use of the Lantern for Projecting Photo-Micrographs on the Screen.

The sensitive plates, used for making the lantern slides, are coated with a gelatine bromide emulsion which, when exposed to light through a negative, and developed according to directions, will give an attractive positive firmly adhering to the glass. Lantern slides of a high grade of excellence may also be made by the use of the "wet plates," which of course must be coated with the sensitive emulsion just before using. This is the old method, and is well understood by the professional photographer, and was the only method for making slides before the "dry plate" came into such general use. It requires some experience, however, to prepare the plate properly, and there is more trouble in manipulating it in the hands of the amateur. When carefully handled the dry plates give brilliant and fine results, and they are to be recommended to the student.

The dry plates are sent out by the manufacturers in light-tight boxes, one dozen in each box. The operator should obtain a negative box the proper size, and fill it with the plates after they have been dusted with a camel's hair brush. This transfer must, of course, be done in the dark-room. The negative box will hold twenty-four plates.

The lantern slides are made by either one of the following methods:

1. The contact method.
2. Photographing the negative by means of a camera.

The first method is only feasible when the negative

has been made near the size of the picture to be transferred on the positive glass, and the work is accomplished as follows: Mix a sufficient quantity of the developer, in accordance with instructions contained on page 141, light the lamp and exclude all rays from the dark-room except those which come through the ruby glass of the lamp. Place a negative, film side up, in a deep printing frame, take one of the sensitive plates from the box and lay it on the negative *with film in contact with film*, place a piece of thick felt on the back of the sensitive plate to exclude all reflected rays of light from the rear of the printing frame, and then put on the hinged back and clamp it into place. Now stand the frame on edge about 18 or 24 inches from the lamp with the negative towards the light, cover the frame with an opaque cloth, or interpose a black card, and then open the door of the lamp so that the white light many be projected towards the printing frame, raise the cloth or cover for a few seconds (the time depending upon the distance between the light and negative, strength of light and density of negative) until the sensitive plate is sufficiently affected, when the light is cut off. The plate is now transferred to the developing pan with the film side up, and the developing solution quickly poured over it. If the picture comes up rapidly the exposure has been too long, and another trial should be made. There is but little advantage gained in trying to doctor an under-exposed or very much over-exposed plate. It is cheaper, and best in all respects, to expose another plate, either prolonging or shortening the time of exposure, as the

Figure 82. Printing Frame.

case may demand, until the image begins to make its appearance, not sooner than 20 seconds, and continues to come out gradually and slowly. The development must not be stopped until the high lights just begin to tinge, and all the details of the picture are well out and distinct contrasts exist between the high lights and deep shadows, with gradual blending between the high lights and the deep shadows. The plate is now washed and placed in a fresh fixing bath prepared according to the formula given on page 143. It must remain in this bath until all the white cloudiness has disappeared entirely from the back when the plate can be taken out into the white light and examined with safety. The positive is considered to be first-class if it will show by transmitted light, when held up between the eye and the window, perfectly clear glass for the high lights and passing by imperceptible gradations to the deeper shadows; and if the tone is of a warm purplish black. The deep shadows must not be so opaque as to prevent the outlines of the flame of a lamp from being seen when the slide is held between the observer and the light. Carefully wash the slide in the same manner described for the negative and swab off gently with a wad of soft cotton to take off all extraneous particles which may be adhering to the film, and stand in a rack to dry.

To make the slide by the second method, or photographing through the camera, it is necessary to have a camera with an extra long bellows. The negative is suspended in front of a window with a northern exposure, if possible, and the camera is carefully adjusted at a suitable distance from the negative so that the image projected on the ground glass plate will be the desired size for the slide. The front of the camera, should be parallel with the surface of the negative and the optical axis of the lens must strike the centre of the picture. If the negative is smaller than desirable reverse the lens of the camera and the enlargement of the image is produced on the ground glass. A lit-

tle ingenuity in manipulating the apparatus will soon produce the results sought for by the operator. As soon as all portions of the apparatus have been properly adjusted the distance between the negative and the camera is covered with a dark opaque cloth to exclude all light except that which comes directly through the negative, and the operator is then ready to substitute the plate holder containing the sensitive plate for the ground glass and make the exposure. Before pulling out the slide covering the plate see that the lens has the proper diaphragm and is covered with a cap. The experience of the author has demonstrated that the diaphragm f 16 and the light coming from a clear northern sky, with Carbutt's "geletino-albumen" plate, 15 to 20 seconds, will yield brilliant lantern slides. There are the same factors, however, to be considered here which were mentioned in the case of the time of the exposure in making the negative. (See page 140.) The experience of the operator must determine these questions after he has become familiar with the qualities of his negative and the character of the light.

The after treatment of the plate is, in all respects, the same as that described under the first, or contact, method. When the slide is thoroughly dry after washing, the film should be covered with a suitable varnish, made for the purpose (usually colorless gums dissolved in benzole), so that moisture cannot injure the slide in the future. Moreover, the varnish benefits the slide in cleaning it up and brightening the picture throughout.

To mount the slide for permanent preservation, take a thin glass plate, perfectly free from all flaws and well cleaned and cover the film. Between these two glass plates insert the mat illustrated in figure 83, and then bind the plates together by means of a narrow strip of gummed paper three-eighths of an inch wide and fifteen inches long. Lay this strip on the table and wet with a dampened sponge three or four inches at one end. Take the slide with the cover-

glass and mat in position, and press one of the edges in contact with the wet slip; invert the slide, resting the opposite edge on the table, and, by means of the fingers, bring the damp portions of the slip in close contact with the glass.

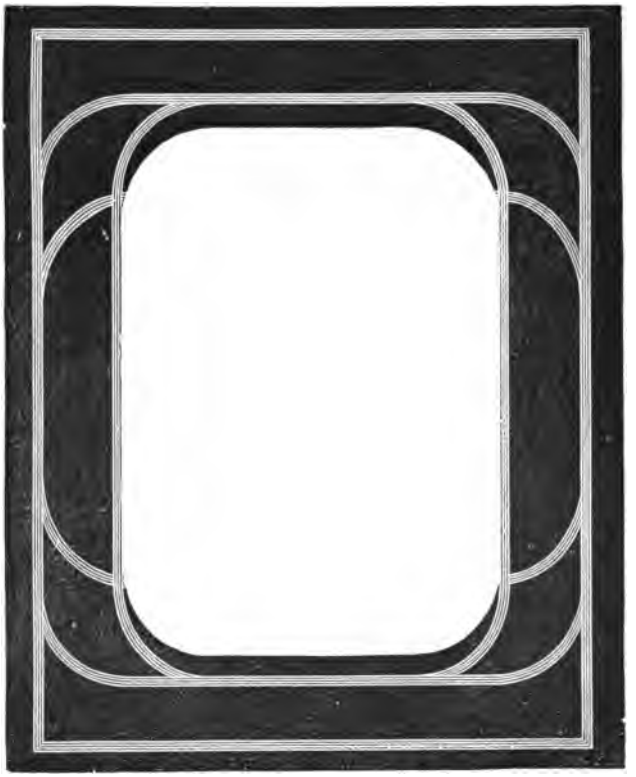


Figure 83. Mat for Lantern Slide

Proceed to dampen the gum and adhere the slip until all four edges of the slide have been covered. While performing this operation, keep the fingers dry, if good work

is desired. The label is placed on and then the slide is ready for the lantern.

Figure 84 is an excellent box for holding the slides while they are being put through the lantern, and it will also serve a fine purpose in preserving them from the action of dust, when stored away.

Figure 84 Box for Lantern Slides.

The Lantern and its Requisites.—The lantern consists of the following parts, which are absolutely essential to its well working:

1. The radiant or lamp.
2. The condensers, for collecting and concentrating the light on the slide.
3. The slide carrier.
4. The body of the lantern, which consists of a metal box or an extension bellows.
5. The projecting system or lens for projecting the rays which pass through the slide on to the screen and produce the enlarged picture.

The description of the light suitable for the lantern has

already been given in Chapter IX., so the student is referred to that part of the book for information on that point. What is true in the case of illuminating the object for projecting through the objective, is equally true in regard to the character of light for lantern purposes. Figure 67 is by far the best lamp for the lantern with which the author is acquainted. However, in figure 85 is shown a lantern lighted with the oxy-hydrogen lamp, after the Colt's pattern, which is given as an illustration of a most excellent method of illumination in the hands of those who prefer the oxy-hydrogen light to the electric lamp. Fig. 86 is also given to show the position of this lantern when attached to the oil lamp. This oil light will yield fairly good results when used in a small room with the screen very near the lantern. Figure 87 shows the lantern with the projecting lens replaced with the microscope attachment when it is intended to project the image of the object directly without first making the photographic slide.

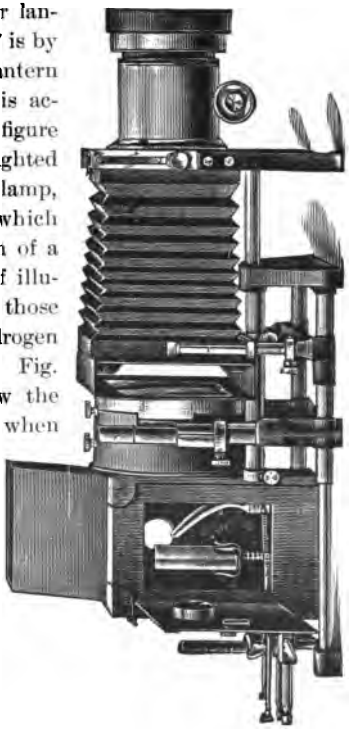


Figure 85. Colt's Criterion Oxy-Hydrogen Lantern.

The condensers are two plano-convex lenses about four inches in diameter, so mounted that the convex sides are towards each other. One of the best arrangements of condensers is shown in figures 90 and 91; it is so

constructed that the back lens, or that one nearest the light, is hung on springs, thus permitting of expansion and at the same time allowing the air to circulate between the two glasses, which is a great advantage.

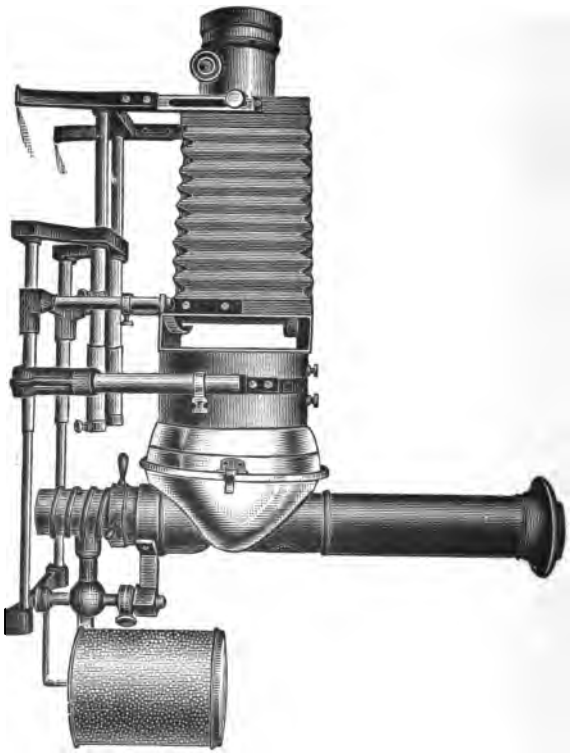


Figure 86. Colt's Criterion Oil Light Lantern.

Where there are two lanterns in the laboratory beautiful effects are secured by producing what is termed dissolving views. This is accomplished by placing the lanterns near each other so that the beam of light thrown from each will cover the same portion of the screen; and, if the oxy-hydrogen jets are used, the key illustrated in figure 92 is so connected with the oxygen and hydrogen

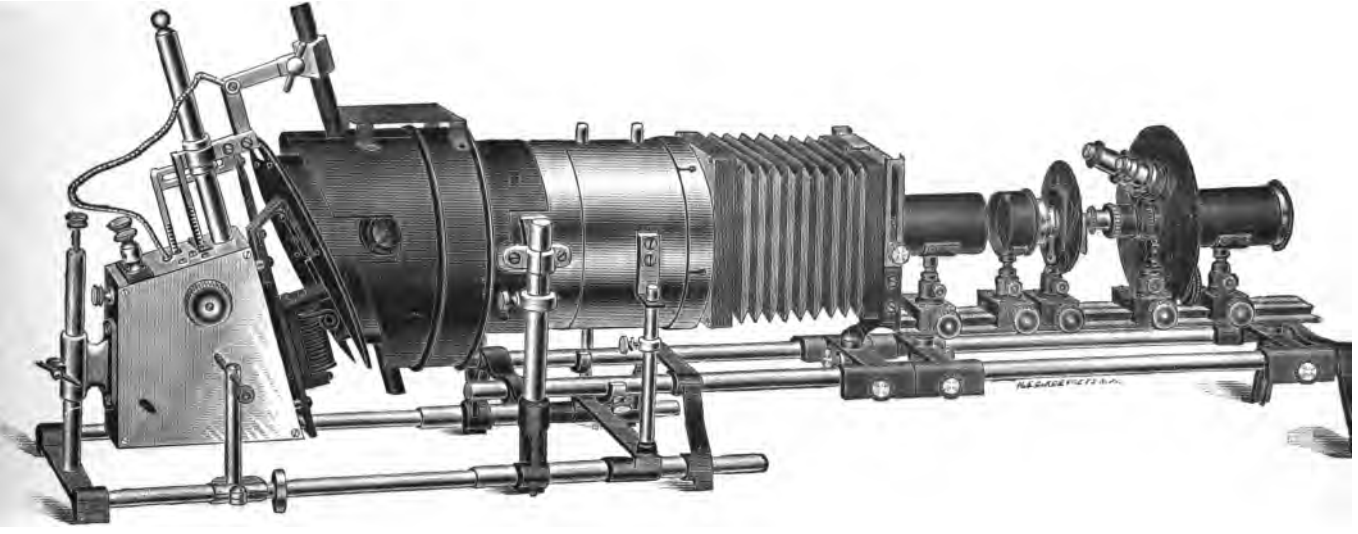


Figure 87. Colt's Criterion Lantern with Electric Lamp and Improved Microscope Attachment

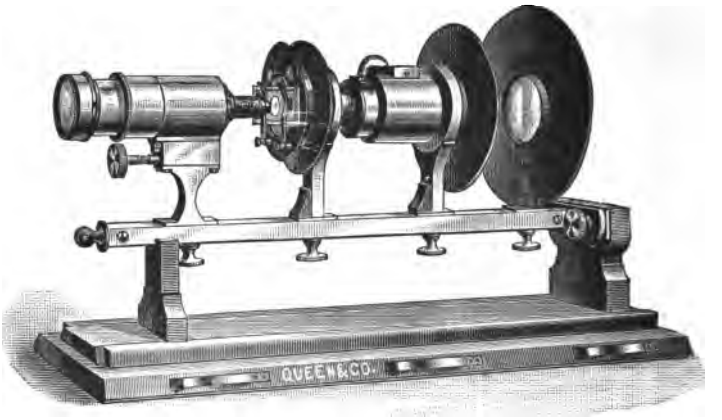


Figure 88. Queen's Microscope Attachment for the Lantern.

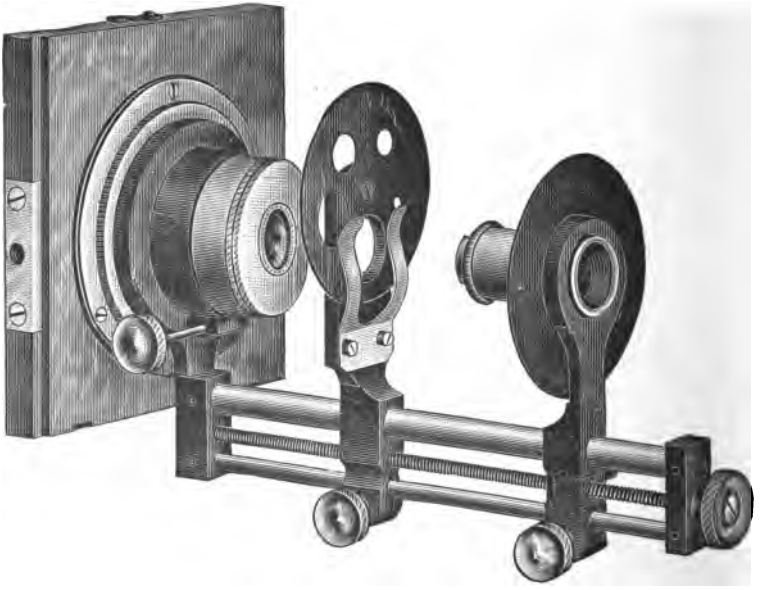


Figure 89. Colt's Microscope Attachment for the Lantern.

Figure 90. Colt's Improved Condenser.

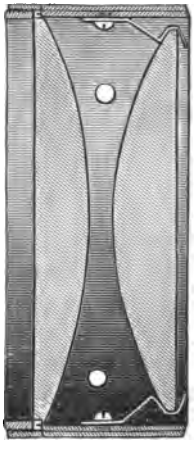


Figure 91. Colt's Condenser—Cross Section.

cylinders that the gases will pass through the key and may be transmitted first to one of the lanterns and then to the other at the will of the operator. The effects on the screen are very beautiful when one picture dissolves off as the other grows into full strength. In the case of the electric lamp the dissolver shown in figure 93 must be used. This key is placed in front of the lanterns and the effects are just as pleasing as in the case of the last one described.

The Care of the Lantern.—Like all other delicate apparatus the lantern is liable to serious damage unless carefully protected from dust and sudden changes of temperature. When the lamp is lighted the flame should be kept rather low until the condensers are gradually

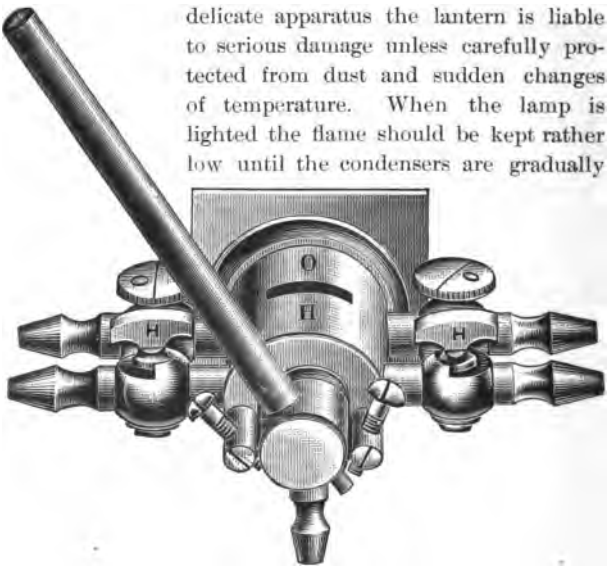
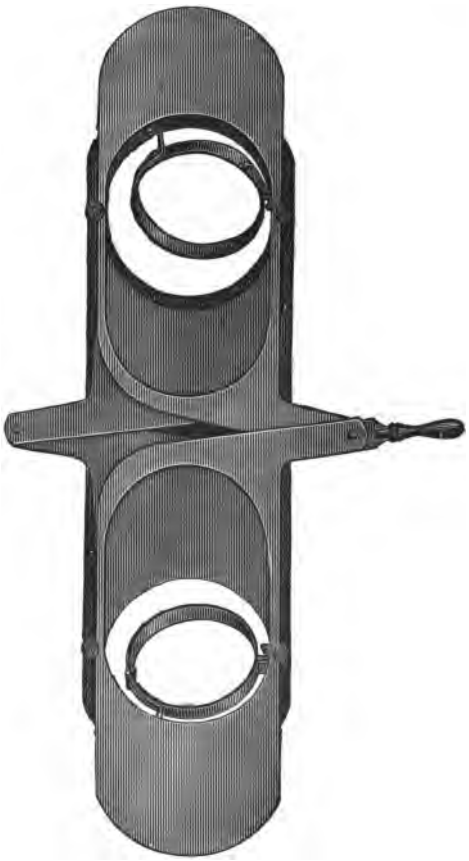


Figure 92. Colt's Dissolving Key for Oxy-hydrogen Lamps.

heated. For this purpose it is best to turn on the light ten or fifteen minutes before the pictures are projected on the screen, and then the light can be raised to its fullest intensity without so much danger of breaking the condensers and other glass lenses. An excellent protection against flying particles of highly heated lime and carbon is a piece of clear mica interposed between the light and the lenses.

The same care should be manifested in turning off the light to prevent the glasses from cooling too quick, because the unequal and rapid contraction will often cause them to



IMPROVED MECHANICAL DISSOLVER. For use on any style of Lanterns, giving finer effects than have heretofore been produced.

Figure 93.

break. When not in use the lantern should be carefully covered with a thick cloth to protect all parts of it from

dust. Whenever it is necessary to clean any portion of the glass surface the best material to use is Japanese paper, because it contains no particles of grit and is very soft and has considerable absorbent power for the moisture frequently condensed on the surfaces of the lenses. The rubbing, however, should be gentle that no scratching may be done. *Rub the glass as little as possible;* it is much better to protect them from all particles of dust and sudden changes of temperature.

The light must be centered on the screen before the slide is put in the lanterns; so that a perfectly white light, free from all shadows and colors, will be formed. This is done by adjusting the lamp in three ways: vertically, laterally, and from or towards the condensers. This operation is very quickly accomplished when once learned. It is only necessary thereafter to adjust the front, projecting lenses as each slide may require.

The fact must be borne in mind, in determining the distance between the screen and the lantern, that the nearer the lantern is placed the smaller will be the picture; and, as a general rule, also, the greater the size of the picture the less will be the brightness of the disc of light on the screen.

CHAPTER XII.

Polarization of Light and its Application to Botanical Investigation.

The property of light to polarize, when passed through a prism of a dense, transparent medium, is of great utility in the study of crystallography, but there has been little use made of it in the botanical laboratory in the examination of cell development. In late years, however, some attention has been given the matter by histological botanists, and there is reason to assert that in the near future no laboratory will be considered well equipped without the polarizers in connection with each microscope.

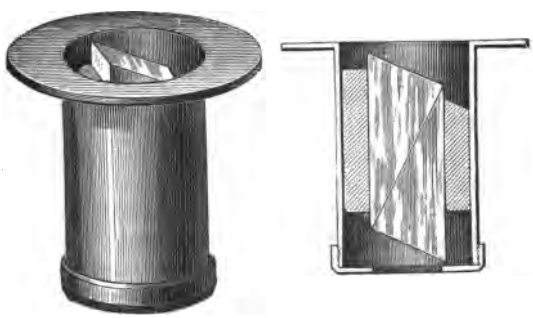


Figure 94. Zeiss' Polarizer.

The parts of the apparatus consist of a polarizer, which is placed beneath the object to be examined, and the analyzer, which is placed above the stage (see figure 94). They contain, each, two Nicol's prisms, so arranged with reference to each other as to reduce the vibration of the particles of ether transmitting the light, into two planes. The Nicol's prisms are two pieces of Iceland spar cut into

rhomboidal shapes and cemented together with Canada balsam. They are properly mounted in tubes, as is shown in the illustration, figure 94, so that they will fit the microscope in the position desired. When the light passes through the first prism it is reduced, as above stated, into two planes, one called the *ordinary ray* and the other the *extraordinary ray;* and when they strike the film of balsam separating the prisms, the ordinary ray is totally reflected while the extraordinary ray passes through the second prism and produces the effect called *polarization.* All light must be excluded from the stage of the microscope except that which comes through the polarizer, because, if this precaution is not taken, the field will not be sufficiently darkened for satisfactory results. When the polarizer and analyzer are in their places on the microscope, it will be found that by turning the analyzer, there is a position when the largest quantity of the light reaches the eye, and also a position when the light is entirely cut off. The first position is when the prisms are parallel to each other, and the latter when they are almost at right angles.

Mohl has done much valuable work with the polarizers in investigating the effects of the light on vegetable organisms, and Messrs. Griffith and Henfrey thus sum up the results secured by him: [1]

"It is easy to ascertain whether an organic body shows positive or negative colors, by comparing its color, when seen with a plate of gypsum in a certain definite position, with the color given under the same circumstances by a strip of glass brought into a state of tension by a slight bending, or with the colors of a suddenly cooled globule of glass. In this way the author determined that the fibres of a spiral vessel displayed negative colors, and the laminæ of a starch corpuscle positive colors, and then applied these organic structures, by comparison, for ascertaining the properties of other objects. The objects to be examined

1 Micrographic Dictionary, Griffith & Henfrey. Pages 612-13.

should be mounted in a liquid or other substance rendering them as transparent as possible, such as glycerine, Canada balsam, or an essential oil.

"When ordinary globular or cylindrical cellular tissue are viewed by cross sections, their substance is seen to be doubly refractive; for when the prisms cross, the circular sections of cell walls appear like rings of bright light on a black ground, but with the ring divided into four quadrants by dark stripes, as if a black cross lay over it; when the prisms are placed parallel, the parts of the section previously bright appear dark, and *vice versa*, on a bright field. If a section of polyhedral cellular tissue is viewed in the same way, the appearances are somewhat different, since the cut edges are here straight lines, variously inclined towards the prisms; those which are perpendicular to the prisms are invisible, while those standing obliquely are bright in their whole length. In general, cell-membrane acts more powerfully on the light the denser its substance, and soft collenchymatous tissues are far less powerfully doubly refractive than wood-cells. When the cells have walls. much thickened, it is common for the primary cell-membrane to be much more powerfully refractive than the secondary layers.

* * * * * * * * * * * *

"Let us suppose that between the power prism and the object is placed a plate of selenite giving a red field; the plate is then rotated so that its neutral axes are at an angle of 45° with the prisms. A section of a cylindrical vegetable cell will be seen to be divided into four quadrants: the two alternate quadrants, whose middle lines correspond to the neutral axes of selenite, are either blue or green, the other two yellow or red; if the selenite is then rotated so that its neutral axes are perpendicular to the prisms, the colors will be all lost; but on continuing the rotation, they appear in the reverse order—what was blue appearing yellow, and *vice versa*. When the walls are rectilinear, all the cell-walls perpendicular to one of the prisms will give the color of the

field, all those which run parallel with one of the neutral axes of the selenite plate, or form no great angle with it, will be blue, those parallel with the other axis yellow.

"It is found that the vegetable structures fall into two classes in reference to these colors, in one of which classes all layers lying obliquely in the direction of a right-wound screw are tinged blue and yellow, those oblique in the opposite direction yellow or red; in the other class, the colors under the same conditions are just the reverse; so that one class are optically positive, the other optically negative.

"The optically negative are the ordinary cell-membranes of the internal organs of plants, whether in their natural condition or cellulose purified by the help of nitric acid and chlorate of potash: Collenchyma, horny endosperm-cells, the gelatinous cells of Algae, etc., all agree in this property. Optically positive colors are given by cell-membranes of periderm and cuticular layers of epidermal cells. The contrast of the positive and negative colors of the cuticle and other parts of the cell-wall is well seen in the epidermis of *Aloe*. The diversity of coloring under polarized light here corresponds to the diverse behavior under treatment with iodine after maceration in solution of potash (secondary deposits).

"The longitudinal sections of all behave like the cross sections; but the appearances are not so clear. When side views of the surface of cells are obtained, the phenomena are very varied; but these are not best seen in vessels or ducts when the thickening layers are in the form of spiral bands. Thus, if one of the spiral vessels of *Musa* is placed (its spiral somewhat drawn apart) with its long axis perpendicular to one of the prisms, the fibres on the upper side turn to the left, those on the under side towards the right; and when the selenite plate is interposed, they exhibit the complementary colors. When the side walls of cells have obscure striation, as in the cells of

conifers, the liber-cells of Apocyneae, &c., the membrane give evidence of its fibrillar structure by the yellow or blue color developed with the selenite plate. If fibres of a spiral vessel cross at right angles, and they are pressed together, they neutralize one another when they cross: when the prisms are used alone, the crossing points are black, the rest of the fibres white; when the selenite plate is interposed, the crossing points exhibit the color of the field, and the uncrossed portions of the fibres are blue or yellow according to position.

"The vicinity of a round bordered pit, as in the wood-cells of *Pinus*, exhibits a black cross when seen perpendicularly by polarized light. The black cross and the colors exhibited by starch are well known. Chlorophyll does not seem to act on polarized light, nor the primordial utricle of cells, except a trace when contracted by weak alcohol.

"The polarization apparatus is exceedingly useful for the detection of crystals (Raphides) in vegetable tissues, when they are so small as to be readily overlooked; and the larger kinds form beautiful objects with, and often without the selenite plate."

By an elaboration of these experiments with sections of various kinds of plants, much useful information will be gleaned, which will enable the microscopist to arrive at conclusions in no other way attainable. The limit and the character of this work will not permit of more extended discussion of this interesting subject, but the director of the laboratory will give the students much valuable as well as interesting, information if he will require them to make frequent tests with the polariscope, while studying cross sections of the plants. For a full account of polarization in microscopical investigation, the student is referred to the writings of Dippel, Nageli and Schwendener.

CHAPTER XIII.

Useful Formulæ and Tables.

There are many useful formulæ and tables which are of importance to microscopists, scattered through the pages of journals and books devoted to botanical research, and their enumeration would much more than fill the leaves of a book larger than this. The attempt, therefore, will be made to mention only a few of the more important:

Cleaning Glass.—1. Place in a wide-mouth bottle a strong solution of borax and washing soda, or caustic potash. The glasses placed in this will soon become cleaned of gums and other foreign matters.

2. Dichromate of potash.....10 grammes.
Hot water.....50 cc.
After cooling, add slowly sulphuric acid..50 cc.

First remove the covers from slides by heating two or three seconds over Bunsen's burner; place the glasses in a porcelain dish, pour over them the fluid and heat on water-bath for ten minutes. When clean, wash the glasses and immerse in solution of dilute caustic soda, warm for five minutes, place in spirits and dry.

3. "One box or pound of crude potash dissolved in one gallon of water. Bring to a boil. Dip the old gelatine plates in this, in a dipping rack that will keep them apart. Dip with movement, and when the film is gone remove the rack and glass into another vessel of warm water, and then wash well in running water. Take them, one at a time, and rub with rag covered with pulverized cuttle-fish bone. Put them in a rack to dry and then pack away for use. When the plates are to be used polish off the fish bone and dust with a piece of soft linen."—*Holmes and Griswold.*

4. Dr. F. Knauer's method: The slides are placed in a

vessel containing about half a litre of 10 per cent. solution of lysol and boiled for twenty to thirty minutes. The still seething vessel is then placed under the faucet and the water allowed to flow in until the solution becomes quite clear, when the slides are taken out and dried.

5. A vessel containing a lump of washing soda and water brought to boiling, will remove all the gum from slides and cover-glasses, so that the latter are easily recovered.

Stains.—6. Dr. E. Vinassá, in "Zeitschrift fuer wissenschaftliche Mikroskopie," divides anilin stains into three groups:

(1) Those which stain only the parenchyma;

(2) Those which stain the lignified tissues, collenchyma, vessels, nuclear, sheathes, etc.;

(3) Those which merely differentiate—that is, render more conspicuous the thickened cells from the surrounding parenchyma (which latter, of course, is lighter colored than the former).

The following is the list of stains examined with the results noted:

(1) Safranin T, (2) Vesuvin, (3) New Victoria green extra, (4) Light green (yellowish green), S. F., (5) Victoria Blue B., (6) Ponceau RRR., (7) Chrysoidin A., (8) Chrysoidin P., (9) "Echtroth" red A., (10) Safranin, (11) Phloxin, (12) Crystal violet BBBBBO. (13) Congo red, (14) Cyanosin, (15) Victoria blue RRRR, (16) Orange 11, (17) Violet B., (18) Phosphin, (19) Victoria blue BB, (20) Roccellin, (21) Solid green crystals, (22) Auramin O., (23) Benzopurpurin BBBB, (24) Deltapurpurin, (25) Methyl green, (26) Diamondfuchsin G., (27) Erythrosin, (28) Bordeau, (29) Methylen blue G., (30) Eosin, (31) Benzopurpurin B., (32) Benzopurpurin RRRRRR., (33) Chinolin yellow, (34) Rosazurin G., (35) Congo corinth B., (36) Hessian yellow, (37) "Tuchroth" red B., (38) Hessian purple, (39) Naphtharubin, (40) "Echtroth," (41) Chrysamin 11, (42) Chrysophenin, (43) Azoviolet, (44) Azoblue, (45) Chrysamin 1, (46) Brilliant yellow, (48) Curcumin, (49) Benzo-azurin R., (50) Metanil-yellow, (51) Chrysoidin nitrate crystals.

Stain parenchyma: 1, (5), 13, (15), (19), 23, 24, 31, 32, 34, 41, 42, 43, 49.

Those in parenthesis are difficult to wash out, but excellent for differentiation (group 3).

Stain thickened cell-walls, lignified tissue, etc.: 2, 3, 4, 5, 6, 7, 8, 9, 12, 15, 16, 18, 19, 20, 21, 22, 25, 26, 28, 29, 33, 37, 39, 40, 46, 48, 51.

Stain thin-walled cells: 35, 36, 38, 44, 45, 47.

Stain vessels: 1, 10, 11, 14, 17, 21, 27, 30, 50.

Easily washed out: 6, 9, 11, 16, 20, 28, 30, 40.

Precipitated by acids: 5, 9, 11, 13, 14, 15, 16, 17, 19, 20, 23, 27, 30, 31, 34, 35, 36, 37, 42, 43, 45, 47, 48.

Precipitated by alkalies: 1, 2, 3, 5, 7, 10, 12, 13, 15, 17, 18, 19, 21, 22, 24, 25, 29, 42, 46.

Not altered by acids: 2, 6, 7, 28, 29, 39, 40, 49.

Not altered by alkalies: 11, 14, 23, 27, 30, 31, 32, 34, 41, 50.

Not precipitated by acids nor by alkalies: 4, 5, 8, 26, 28, 32, 33, 38, 39, 40, 41, 49, 50, 51.

(Of course, the color or shade will in all instances be more or less influenced by either acids or alkalies.)

For double-staining, Vinassa especially recommends: (21) or (25) and (24); (7 or 8 or 51) and (49); (29) and (23 or 24 or 31 or 32).

The following colors require to be fixed: With tannin: 1, 2, 10. With acetate of lead: 11, 14, 27, 30. The remainder of the colors are stated to be "subjective."—*The Microscropical Bulletin, December, 1891.*

7. Staining of Protoplasts and cell-walls: (Herr J. af Klercher, Royal Mic. Soc. Jour., 1893, p. 562, and Verhandl. Biol. Ver. Stockholm, lv., 1892, No. 14): "If the object examined is an ærial part of a plant, the oily substances are first removed by ether or dilute ammonia, and, after washing out the fixing material, the object is allowed to somewhat dry in order to promote the entrance of the staining substance. But if it is only the membrane which is to be stained, the object is brought directly, or after washing out the fixing material, into eau de Javelle or eau de Labarracque, and left there till all the protoplasm is

dissolved. After careful washing, it is then stained with a moderately concentrated solution of congo-red, and finally, after careful washing, placed in paraffin. A good staining of membranes may also be effected by successive treatment with iron salts and potassium ferrocyanide, or with tannin and ferric chloride."

8. An aqueous solution of hæmatoxylin which does not readily deteriorate:—*S. H. Gage, Cornell.*

Distilled water	300 c. cm.
Potash alum	10 grammes
Chloral hydrate	6 grammes
Hæmatoxylin crystals	$\frac{1}{10}$ gramme

Boil the water with the alum in a porcelain dish for five minutes. When cool add the chloral hydrate and the hæmatoxylin. It is advantageous to dissolve the hæmatoxylin in 5 to 10 c. cm. of absolute or 95 per cent. alcohol before adding to the alum solution. The color will be light at first but will darken materially in a week or so. The object of boiling the alum solution is to destroy all germs, and the addition of the chloral hydrate is to prevent their reappearance. The solution may be rendered more concentrated by the addition of more hæmatoxylin. Mixture of alum chloral and water make the best diluent.

9. Alum Carmine: (Boneval. See Amer. Mic. Jour., 1893. Page 78.)

Ammonia alum	1 to 5 grammes
Carmine	4 grammes
Distilled water	100 grammes

"Boil for twenty minutes, taking care to maintain the original volume by adding water. Filter and preserve by a crystal of thymol. It is a nuclear stain of the first order; it colors admirably the nuclei of tissues fixed by osmic acid, which makes it valuable in many instances where picrocarmine is worthless. The color is well preserved in glycerin. Place the sections for a few hours in a vessel containing one c.c. of alum carmine, and wash until the excess of color is removed. This stain is exceedingly penetrating, so that tissues may be colored in mass."

10. Rosanilin violet: (Hanstein's Practical Botany,

Strasburger, p. 398). "Equal parts of methyl-violet and fuchsin (majenta), mixed and dissolved in alcohol. Shows stratification of cell-walls, and differentiates sections of stems, especially monocotyledons. Stains protoplasm bluish-violet; amyloid substances, nucleus, and gums different shades of red; resins blue; tannin, foxy-red; cellulose, pale-violet; lignin, reddish; bast-fibres, deep red; sieve-tubes, and bast parenchyma, hardly at all."

11. Mayer's Paracarmine alcoholic stain (The Mic. Vade-Mecum, Lee, page 106):

Carminic acid	1 gramme.
Chloride of aluminium	0.5 gramme.
Chloride of calcium	4 grammes.
70 per cent. alcohol	100 c.c.

Dissolve cold or warm, allow to settle and filter. Object to be stained should have an alkaline reaction.

12. Picro-carmine (Ranvier's. See Lee's Mic. Vade-Mecum, page 102):

Water	1000 parts.
Picric acid	20 parts.
Carmine	10 parts.
Ammonia	50 parts.

Put the chemicals in a stoppered bottle and leave in a warm place for two to three months. Place in an evaporating dish and allow to crystallize; if the crystals will dissolve in warm water and after filtering the carmine is found really in solution, evaporate to dryness and reduce the residue to powder. For use, dissolve 1 gramme in 100 grammes of water, and add crystal of thymol to preserve it from formation of mould.

13. Beale's Carmine:

Carmine	0.6 grammes.

Dissolved in boiling solution of ammonia. Let stand for an hour to cool and to permit superfluous ammonia to escape. Add to the solution:

Water	60 c.c.
Glycerin	60 grammes
Absolute alcohol	15 grammes

Permit to stand for some time and filter.

14. Anilin-violet: (Hanstein's Practical Botany, Bower.

Page 331). "Dissolve equal parts of fuchsin and methyl-violet in alcohol. It stains cellulose cell-walls of a faint violet color, and lignified cell-walls deep violet. It is especially useful for bringing out the different parts of the bast, since the bast-fibres stain red, whereas the sieve-tubes and the parenchyma scarcely stain at all. The protoplasm is stained pink: amyloid substances, gums and nuclei stain different shades of red, resins blue, and tannin brick-red."

15. Differential Nucleolar Staining: (Gustave Mann. See Jour. Roy. Mic. Soc., 1891. Page 690). "Tissues, both vegetable and animal, preferably fixed by picro-corrosive method (Mann's), are treated for ten minutes in a saturated solution of heliocin in 50 per cent. alcohol; the sections are then transferred for from five to fifteen minutes to a standard watery solution of analin blue. The supurfluous stain is rapidly washed off by distilled water, and the sections placed again for one or two minutes in the heliocin-solution, dehydrated, cleared by resinified turpentine, and mounted in turpentine balsam. The whole of the cell and the nucleus (is stained) blue, the nucleolus red."

16. Staining of Chlorophyll: (Mann. See Jour. Roy. Mic. Soc., 1891. Page 689.) "A glass vessel is filled with two litres of water, to which six drops are added of a 10 per cent. solution of cyanin in absolute alcohol. Then a small quantity of either *Spirogyra jugalis or S.nitida* is placed in the vessel, which is exposed to the bright daylight. After some time, varying with the temperature of the room and the activity of the threads, from three to twenty-four hours, the whole of the cyanin will have been taken up by the threads. The ground-subtance of the chlorophyll-bands will have changed from a green to a bluish-green color, while the oil-globules and many of the microsomes between the bands will have turned blue, showing their fatty nature."

17. Staining Cell-nucleus of Pollen-grains: (Herr A.

Meyers's method. See Jour. Roy. Mic. Soc., 1892. Page 899.)

Carmine..... 0.5 grammes
Absolute alcohol.....20 c. cm.
Hydrochloric acid.....30 drops
Heat for thirty minutes in water bath and add
Chloral hydrate.....25 grammes

Filter and cool. Stains nuclei of pollen-grains within ten minutes an intense red.

18. Staining paraffin sections: (Amer. Mon. Mic. Jour., XI, 1890. Page 11.) Dissolve the coloring matter in absolute alcohol and drop the solution in turpentine until the desired depth is secured. Sections fixed to the slip by the collodion method are placed in the oven until the oil of cloves is completely evaporated, the paraffin is dissolved in turpentine and the slide is then put in the stain. The action is quick. Over-staining may be corrected by placing in a solution made with equal parts of acid, free absolute alcohol and turpentine. Meyer's carmine, methyl-green methyl-blue, gentian-violet, safranin, Bismarck-brown, eosin fuchsin may be used as indicated with good results.

(The student is referred to Lee's Microtomist's Vade-Mecum for full information on all kinds of stains and other formulæ used in mounting and preserving specimens of animal and plant organization.)

Cements and Mounting Media.

19. Canada balsam: Heat the solid balsam until brittle when cold and then dissolve in either benzol, xylol, chloroform or turpentine to the required consistency. The xylol solution is generally considered to be the best, but it sets more slowly than the benzol solution.

20. Damar: Dissolve without heating in xylol, or one of the other menstrua mentioned in (19).

21. Glycerin jelly: (See Lee's Mic. Vade-Mecum and Frey's Le Microscope).

Glycerin.....120 grammes
Water..... 60 grammes
Gelatin..... 30 grammes

Dissolve the gelatin in the water and add the glycerin.

22. Farrant's Medium:

Picked gum arabic.....4 ounces.
Water .....4 ounces.
Glycerin.....2 ounces.

To be kept in a stoppered bottle with a lump of camphor. See also Jour. Roy. Mic. Soc., 1890, page 820.

Fixing and Clearing Formulae.

23. Mayer's albumen fixing method (Lee):

White of egg.....50 c.c.
Glycerin.....50 c.c.
Salicylate of soda.. ..... 1 gramme.

Shake well together and filter into a clean bottle.

The following is a modification of Mayer's method, given in the American Monthly Mic. Journal, page 119, 1895:

"A layer of Mayer's albumen was spread on the slide and the sections arranged. Then a wash ¼ per cent. collodion was spread over the surface evenly with a camel's hair brush. This is allowed to dry, which takes place in about one minute, but a longer time does no harm; practically one slide dries while the next is being prepared. During the drying many small air bubbles appear, the presence of which indicates the right degree of dryness; these do not cause any inconvenience, as they disappear during the subsequent processes. When dry, the slide is put up *without heating*, into a jar of xylol or benzin for half an hour or more, to dissolve the paraffin. A stay of several hours will not injure the tissues. The paraffin may be removed in three to five minutes by constantly moving the slide in the benzin. The benzin or xylol is removed by 95 per cent. alcohol, and the sections are stained and mounted as desired."

Preserving Medium for Plants.

24. Formalin or formol preserving medium:

Formaldehyd ..... 40 parts.
Water .....100 parts.

Chlorophyll and all delicate parts of plants are well preserved in this solution, and the colors are not destroyed—some flowers are preserved.

25. Used at Paris Museum of Natural History for preserving specimens of plants in original colors:

30 grains of salicylic acid to one quart of water.

Miscellaneous Receipts.

26. Dead black:

Ivory or lamp black 2 grains, a drop or two of gold size, thoroughly mix and add 24 drops of spirits of turpentine. Apply with camel's hair brush to produce a fine coating of black.

27. Black polish on brass:

1. Nitrate of silver..... 1 ounce.
   Water.. ... .20 ounces.
2. Nitrate of copper..... 1 ounce.
   Water .....20 ounces.

Mix the two solutions together and dip the brass into it and heat in an oven until the degree of black is secured.—*National Druggist.*

Another receipt for blackening brass: Clean from all grease, cover with a solution of nitrate of copper and apply heat. May be lacquered by applying shellac varnish and gently heating.

28. To keep metallic objects from rusting:

White wax .....11 parts.
Suint..... 2 parts.

Dissolve in spirits of turpentine.

29. Writing on glass:

Shellac .....2 parts.
Venice turpentine.....1 part.
Oil of turpentine..... .....3 parts.
Lamp black.....1 part.

Dissolve in water bath.

30. Another:

Melt together spermaceti four, tallow three, and wax two parts; and add six parts of either red lead, white lead, or Prussian blue, according to color desired. The mass is turned out in sticks for use.—*Popular Science News Formulæ or Faber's.*

31. The so-called diamond ink, for writing on glass, consists of a mixture of fluoric acid and barium. The latter

is used as a body for the acid. Permit to remain on the glass for fifteen minutes and then rub off the barium, and the characters will be impressed in the glass.

32. The emission of pollen tubes:

Dr. B. D. Halsted recommends that pollen grains be placed in a 10 to 70 per cent. solution of sugar, and the pollen grains will produce tubes within a few hours.

34. Absolute alcohol: Add anhydrous cupric sulphate to 95 per cent. alcohol. The pulverized cupric sulphate is first heated to red heat to drive off the water of crystallization, and, after cooling, is shaken in the alcohol and the mixture allowed to stand a day, the liquid decanted, fresh cupric sulphate added, and the operation repeated until the blue color due to the presence of water is almost, if not quite destroyed. To test, treat a drop of the alcohol with a drop of turpentine on a slide and examine under the microscope to detect particles of water.

35. Softening dried material:

Caustic potash	1 part
Glycerin	5½ parts
Water	5 parts

36. Schulze's mascerating fluid: This is used for separating the parts of woody tissue and is prepared by dissolving in 50 c. c. of nitric acid 1 gramme of potassium chlorate. Used in small quantities, and metallic instruments must be proteced from its effects.

37. To clean the hands of cements:

Castile soap, shaved fine	15 parts
Alcohol, 95 per cent	10 parts
Benzol, ordinary	10 parts
Ammonia water	5 parts
Glycerin	5 parts

Dissolve the soap in the alcohol, add the ammonia and benzol and after thorough agitation the glycerin. After using the soap wash the hands and finish by rubbing over them a few drops of glycerin.

38. Solution for silvering glass (Burton's):

1. Nitrate of silver.....25 grains.
   Distilled water..... 1 ounce.
2. Potash C. P.....25 grains.
   Distilled water..... 1 ounce.

A.

Equal parts of solution 1 and 2. Ammonia to just dissolve precipitate; solution 1 to just cause coloration.

B.

Loaf sugar	2,700 grains
Distilled water	20 ounces.
Nitric acid	2 drachms.
Alcohol (strong)	10 ounces.
Distilled water to make up to	80 ounces.

For use:

Solution A	1 ounce.
Solution B	1 ounce.

Solution B improves with age, while A deteriorates.

39. Ground glass varnish:

Sandarac	90 grains.
Mastic	20 grains.
Ether	2 ounces.
Benzol	½ to 1½ ounces.

The proportion of the benzol determines the character of the surface.

40. To clean a negative stained with silver: Rub with cotton moistened with a solution of cyanide of potassium, wash well and dry.

41. Size of photographic glass and mounts:

Petite	1⅝ x 3⅛ inches.
One-ninth plate	2 x 2½
One-sixth plate	2¾ x 3¼
One-fourth plate	3¼ x 4¼
Half plate	4¼ x 5½ and 4¼ x 6½
Whole plate (4-4)	6½ x 8½
Extra (4-4)	8 x 10

42. Silvering glass: (by M. M. Auguste and Louis Lumiere.) "To 100 c.c. of a ten per cent. solution of silver nitrate ammonia is added drop by drop until the precipitate formed is redissolved. Too much ammonia must not be added at first for this might prevent the formation of the

precipitate. The volume of the solution is increased to a litre by the addition of distilled water. This is solution A. Solution B is made by diluting commercial formaldehyde of 40 per cent. with distilled water so as to form a 1 per cent. solution. Solution B can be kept for some time. Two volumes of A are rapidly mixed with one volume of B and the mixture is rapidly poured over the glass to be coated. In five or six minutes, at a temperature of 15° to 19°C, all silver in the solution is deposited in a brilliant layer which can then be washed with water. (Jour. de Physique, Jan. 1895, and Amer. Jour. of Science, June 1895).

Developing Solutions.

43. Carbutt's Pyro Developer:

No. 1 Pyro Stock Solution.

300 c.cm.....Distilled or Ice Water.....10 ounces
1 gramm.....Oxalic Acid.....15 grs.
2 grammes.....Bromide Potass.....30 grs.

Then add Schering's Pyro 1 ounce (30 grammes), and water to make 16 fluid ounces (480 c. cm).

No. 2 Stock Soda Solution.

300 c.cm.....Water.....10 oz.
120 grammes.....Soda sulphite crystals .... 4 oz.
60 grammes.....Soda carb. crys. (or dry grain 1 oz).. 2 oz.
30 grammes.....Potash carbonate..... 1 oz.

Dissolve, and add water to make measure 16 fluid ounces (480 c.cm).

No. 3 Bromide Solution.

14 grammes.....Bromide of Sodium or Potassium ½ ounce
150 c.cm.....Water.....5 ounces

Pyro Developer.

Dilute 2 ounces of stock No. 2 with 7 ounces of water for cold weather, and 10 to 12 of water in summer. To three ounces of dilute No. 2 add 1½ to 2½ drachms (6 to 10 c.cm) of No. 1. The more pyro the denser the negative, and *vice versa.* No yellowing or fogging need be apprehended if our directions are followed. Development should be continued until the image seems almost buried, then wash and place in fixing bath.

For *Instantaneous Exposures* take for a 5 x 8 or 6½ x 8½ plate three ounces of dilute No. 2. Lay the plate to soak in this, and cover pan. Put 2 drachms of No. 1 into the graduate,

and 3 drops of bromide solution. Pour the soda solution off of the plate into the pyro and back over the plate; let development proceed, and examine occasionally. Keep solution in gentle motion over the plate. A *very* short exposure may take ten minutes to fully develop. If the image is not fully brought out this time, add to developer in pan three times its bulk of water, and let plate lie in it covered over for half an hour or more if necessary, until full development is attained, then wash and proceed as directed under head of developer.

44. Eikonogen and hydrochinon developer, for Carbutt's orthochromatic plates, "celluloid" films and transparencies:

Metric Weight	A	Avoirdupois Weight
600 c. cm.	Distilled water	20 ounces
120 grammes.	Sulphite of soda crystals	4 "
22 "	Eikonogen	330 grains
10½ "	Hydrochinon	160 "
960 c. cm.	Water *to make up to*	32 ounces

Metric weight.	B	Avoidupois weight.
600 c. cm.	Distilled water	20 ounces
60 grammes.	Carbonate of potash	2 "
60 "	Carbonate soda crystals	2 "
960 c. cm.	Water to *make up to*	32 ounces

DEVELOPER.

	A	B	WATER
For instantaneous exposures, take	30 c cm | 1 oz	30 c cm | 1 oz	120 c cm | 4 oz
" Landscapes, Sen. 20–27	30 " | 1 "	15 " | ½ "	90 " | 3 "
Full exposures, " 16–20	30 " | 1 "	25 " | ¾ "	120 " | 4 "
" Lantern slides	30 " | 1 "	25 " | ¾ "	120 " | 4 "
Full exposures	and 2 to 6 drops restrainer D to each ounce developer. (See below.)		

NOTE.—More of A will increase density, more of B will increase detail and softness. Temperature of developer should not vary much below 65° nor above 75°. The after-treatment is the same as with any other developer.

45. Carbutt's hydrochinon developer:

A

600 c. cm.	Warm distilled water	20 ounces.
120 grammes.	Sulphite soda crystals	4 ounces.
4 grammes.	Sulphuric acid	1 drachm.
23½ grammes.	Hydrochinon	360 grains.
2 grammes.	Bromide potass.	30 grains.
960 c. cm	Water to make up to	32 ounces.

B

60 grammes	Carbonate potash	2 ounces.
60 grammes	Carbonate soda crystals	2 ounces.
960 c. cm	Water to make	32 ounces.

For proportions see developer above.

C ACCELERATOR.

30 grammes	Caustic soda	1 ounce
300 c. cm	Water	10 ounces

For under exposure add a few drops of above to developer.

D RESTRAINER.

14 grammes	Bromide potass	½ ounce.
150 c. cm	Water	5 ounces.

46. Carbutt's new acid fixing and clearing bath:

4 c. cm	Sulphuric acid	1 drachm.
480 grammes	Hyposulphite of soda	16 ounces.
60 grammes	Sulphite of soda	2 ounces.
30 grammes	[1]Chrome allum	1 ounce.
1920 c. cm	Warm water	64 ounces.

Dissolve the hyposulphite of soda in 48 ounces (1440 c. cm) of water, the sulphite of soda in 6 ounces (180 c. cm.) of water, mix the sulphuric acid with 2 ounces (60 c. cm.) of water, and pour slowly into the sulphite soda solution, and add to the hyposulphite, then dissolve the chrome alum in 8 ounces (240 c. cm.) of water and add to the bulk of solution, and the bath is ready. This fixing bath will not discolor until after long usage, and both clears up the shadows of the negative and hardens the film at same time.

After negative is cleared of all appearance of silver bromide, wash in running water for not less than half an hour, to free from any trace of hypo solution. Swab the surface with wad of wet cotton, rinse, and place in rack to dry spontaneously.

47. Clearing solution to remove yellow stain caused by developer (Carbutt's):

600 c.cm	Water	20 ounces
90 grammes	Sulphate of Iron	3 ounces
30 c.cm	Sulphuric Acid	1 ounce
30 c.cm	Alum	1 ounce

If, after developing and fixing your negative, it is found

1 During cold weather use only half the quantity of Chrome alum in above.

to be stained yellow from the pyro or hydrochinon developer, first wash well to remove all hyposulphite, then immerse in above solution until the stain is removed; again wash well, and dry.

N. B.—It will improve lantern slides to immerse them for a few minutes in the clearing solution after being well freed from hyposulphite.

Intensifying Solution (Carbutt's).

48. *Intensification.* With correct exposure and development, intensification need never be resorted to. The following formula is, however, very effective, and the most permanent of all methods:

No. 1.

16 grammes	Bichloride of mercury	240 grains
16 grammes	Chloride of ammonia	240 grains
600 c. cm	Distilled water	20 ounces

No. 2.

16 grammes	Chloride of ammonia	240 grains
600 c. cm	Water	20 ounces

No. 3. Cyanide-Silver Solution.

180 c. cm	Distilled water	6 ounces
4 grammes	Cyanide of potassium C. P	60 grains
60 c. cm	Distilled Water	2 ounces
4 grammes	Nitrate of silver	60 grains

Pour the silver into the cyanide solution while stirring, and mark bottle POISON.

Let the plate to be intensified wash for at least half an hour, then lay in a 5 per cent. solution of alum for ten minutes, and again wash thoroughly; this is to insure the perfect elimination of the hypo. The least trace of yellowness after intensifying shows that the washing was not sufficient.

Flow sufficient of No. 1 over the negative to cover it, and allow to either partially or entirely whiten; *the longer it is allowed to act the more intense* will be the result; pour off into the sink, rinse, and flow over No. 2, and allow to act one minute; wash off, and pour over or immerse in No. 3 until changed entirely to a dark brown or black. No. 3 can be returned to its bottle, but Nos. 1 and 2 had better be thrown away. Wash thoroughly and dry.

Reduction: In cases of error in development the negative is too intense, the high lights may be safely reduced by the method of Mr. Howard Farmer, viz: Ferricyanide of Potassium (red prussiate of potash) 1 ounce, water 16 ounces, hyposulphite of soda 1 ounce, water 16 ounces; immerse the negative in sufficient hypo solution to cover it, to which have been added a few drops to each ounce of the above Ferricyanide solution; *the speed of reduction depends on the quantity of Ferricyanide present.* When sufficiently reduced, wash thoroughly. To reduce locally, apply the mixed solution to the wet negative with a camel's hair brush to the parts requiring reducing.

Temperature of development has a great influence on the result. It should be kept if possible within a range of ten degrees; between 60° and 70° F. is a good temperature to secure uniform results. Cold retards development; heat accelerates, and causes flatness; therefore in summer time No. 2 alkali solution should be diluted to 3° Baume, and in winter to 5° Baume.

Over-exposure, to remedy in developing: Take ½ ounce No. 1 pyro solution, water 4 ounces, bromide solution 10 to 20 drops. Soak plate in this for two minntes, then add one drachm of No. 2 stock soda solution to start development, and a further addition if required to complete it. An old Hydrochinon developer is excellent for over-exposed films or plates.

Under-exposure can be remedied in a certain degree, but not entirely, by first soaking plate in water to which has been added 1 drachm No. 2 to 4 ounces water, then use developer composed of half drachm each Nos. 1 and 2 to 1 ounce water, and continue development slowly; but the best remedy, when possible to adopt it, is to expose another plate, giving longer exposure.

In very hot weather, we recommend the use of the alum bath *before* fixing and *always* afterwards.

NOTE.—The figures 1 and 2 refer to pyro developer. When hydro or eiko is used read them A and B respectively.

49. Wuestner's Eikonogen developer for Eagle plates:

1. Distilled water.....80 ounces.
   Sulphite of soda crystals..... 3½ ounces.
   Oxalic acid..... 1 drachm.
   Eikonogen..... 2½ ounces.
   Yellow Prussiate of potash..... ½ ounce.
2. Distilled water.....20 ounces.
   Carbonate of potash..... 2 ounces.

Developer: take 4 ounces of No. 1 and ½ an ounce of No. 2.

Fixing Bath.

Water.....100 parts.
Sulphite of soda crystals..... 5 parts.
Sulphuric acid..... 1 part.

And add

Hyposulphite of soda..... 20 parts.

After fixing, place the negative in a 10 per cent. solution of chrome alum; let it remain a few minutes to harden the film, and then thoroughly wash.

50. Cramer's developer:

1. Distilled water.....40 ounces.
   Sulphite of soda crystals..... 2 ounces.
   Eikonogen..... 1 ounce.

Boil for a few minutes. After cooling, pour into a bottle and keep well corked.

2. Water.....10 ounces.
   Carbonate of potash..... 1 ounce.

Developer: Solution No. 1, 3 ounces; solution No. 2, 1 ounce. In hot weather dilute with an equal quantity of cold water.

Fixing Bath:

1. Hyposulphite of soda.....32 ounces.
   Water..... 3 quarts.
2. Water..... 1 quart.
   Sulphuric acid..... ½ ounce.
   Sulphite of soda crystals..... 4 ounces.
   Chrome alum..... 3 ounces.

Pour No. 2 solution into No. 1.

51. Seed plate developer:

1. Hot water.....30 ounces.
   Eikonogen..... 1 ounce.

Thoroughly dissolve and add

Sulphite of soda solution.....10 ounces.

2. Sal soda.....12 hydrometer test.
To develope, take equal parts of Nos 1 and 2.

Fixing Bath:

Alum..... ½ ounce.
Hyposulphite of soda..... 5 ounces.
Water.....16 ounces.

52. Dr. Stolze's amidol developer:

A. Distilled water.....100 grammes.
Potassium metabisulphite..... 25 grammes.
Amidol (Andresen's)..... 5 grammes.

B. Distilled water.....100 grammes.
Potassium bicarbonate..... 20 grammes.

To 100 c.c. of water add 10 c.c. of A, and of B from 5 to 50 c.c., according to time of exposure. For normal exposures, 20 c.c. are sufficient.

53. Metal developer:

A

Metal.....100 grains.
Sodium sulphite..... 2 ounces.
Distilled water to..... 20 ounces.

B

Potassium carbonate..... ¾ ounce.
Sodium carbonate crystals..... 1 ounce.
Potassium bromide.....40 grains.
Distilled water to .....20 ounces.

Dilute with water for less density, and add bromide of potassium in case of over exposure.—*Wilson's Mag., Nov., 1895.*

54. Negative varnish:

Shellac..... 1¼ ounces.
Mastic..... ¼ ounce.
Oil of turpentine..... ¼ ounce.
Sandarac..... 2¼ ounces.
Venice turpentine..... ¼ ounce.
Camphor.....20 grains.
Alcohol.....20 ounces.

55. Developer for lantern slides:

Hydroquinone..... 10 grains.
Amidol..... 10 grains.
Sulphite of soda crystals.....100 grains.
Hot water..... 6 ounces.

The hydroquinone is first dissolved, and the amidol and sulphite of soda added in order given.

56. To produce purple tones in lantern plates which are too warm or too thin from over-exposure: Chloride of gold 1 grain, sulphocyanide of ammonia 15 grains, and water 4 ounces.

An Outline of the Standard Method

covering the process necessary to convert fresh tissues into the finished preparations is thus presented by Dr. Piersol in the *Appendix* to his *Normal Histology:*

1. Fixation of fresh tissue in large quantity by Muller's fluid; renewal when turbid; tissue remains 2–3 weeks.
2. Thorough washing in running water 2–5 hours.
3. Transfer to 70 per cent. alcohol; keep in dark; changs alcohol whenever it becomes deeply tinged, until it remaine colorless.
4. Stain in excess of borax-carmine, 24–48 hours.
5. Transfer directly, without washing, from stain to acid alcohol, 24–48 hours.
6. Wash well in 70 per cent. alcohol, several times renewed, 24 hours.
7. Transfer to 80 per cent. alcohol, 24 hours.
8. Transfer to 95 per cent. alcohol, 24–48 hours.
9. Dehydrate in absolute alcohol, 24–48 hours.
10. Transfer to pure chloroform until tissue sinks, 6–8 hours.
11. Transfer to saturated solution of paraffin in chloroform, 6 hours.
12. Transfer to pure melted paraffin, kept in constant temperature of about 50°C., until all chloroform is driven off, 6–8 hours.
13. Transfer to fresh melted pure paraffin of consistence for imbedding, 10–15 minutes.
14. Imbed tissue in mould; cool rapidly.
15. Section in microtome, first suitably trimming block for cutting.
16. Fix sections to slides by gum or collodion-clove oil.
17. Remove paraffin by benzole, succeeded by turpentine.

18. Drain off excess of turpentine, apply balsam and cover.

19. Place freshly-mounted slide in horizontal position.

20. Clean up and permanently label when thoroughly dry; store in suitable cabinet.

While the duration of the several manipulations as indicated in the above summary represents the time usually required by ordinary objects, yet the individual character of the tissue must be considered in each case, as density exerts much influence on the rapidity with which the fluids penetrate.

When it is desirable to stain the tissue after sections have been cut, the above manipulations must be modified; steps 4, 5 and 6, in such case are omitted and the tissue is at once dehydrated. Removal of the paraffin from the fixed sections of the slides (17) by benzole is at once succeeded by the following manipulations:

a. Transfer to 95 per cent. alcohol to remove benzole, 5–10 minutes.

b. Transfer to clean 95 per cent. alcohol to insure complete absence of benzole, 5 minutes.

c. Transfer to 80 per cent. alcohol, 5 minutes.

d. Transfer to 70 per cent. alcohol, 5 minutes.

e. Stain in borax-carmine solution, 10–15 minutes.

f. Differentiate in acid alcohol (10 per cent.), 6–10 minutes.

g. Wash in 70 per cent. alcohol, renewed, 10–15 minutes

h. Transfer to 80 per cent. alcohol, 15 minutes.

i. Transfer to 95 per cent. alcohol, 15 minutes.

j. Dehydrate thoroughly in absolute alcohol, 15 minutes.

k. Clear sections in oil of turpentine, 5 minutes.

l. Mount in balsam as indicated above in 18.

When hæmatoxylin is used as the stain, the steps *e*, *f* and *g* are omitted, and replaced by—

ee. Transfer to distilled water, 5 minutes.

ff. Stain in properly diluted hæmatoxylin fluid until sufficiently dark, 8–10 minutes.

gg. Wash well in distilled water to remove excess of stain and to differentiate, 10 minutes; then dehydrate by

the ascending series of alcohols included by *h* to *j* as above. —*Mic. Bul. and Science News, April, 1894.*

Miller's scheme for imbedding, sectioning, staining and mounting:

1. When Paraffin is used.	2. When Celloidin is used.
"80 per cent. alcohol. *a*	"80 per cent. alcohol.
Stain. *b*	Absolute alcohol.
Wash.	Alcohol and ether.
Absolute alcohol.	Dilute celloidin.
Clear. *c*	Saturated celloidin.
Paraffin.	Imbed.
Imbed.	80 per cent. alcohol. *f*
Section.	Section.
Fix on slide. *d*	Stain. *g*
Xylol. *e*	Wash (Then glycerin will terminate if used here).
Absolute alcohol.	95 per cent. alcohol.
Stain. *b*	Eosin-alcohol. *g*
Wash.	Oil of origanum cretici; or oil of cloves. *h*
Absolute alcohol.	
Xylol.	
Balsam.	Balsam.

"*a* If sections are to be stained on the slide after imbedding in paraffin, pass at once to absolute alcohol; if the specimen is to be stained *in toto*, pass to stain, etc.

"*b* For staining *in toto* use Grenacheri's borax-carmine, alum-carmine or Delafield's hæmatoxylin. For staining on the slide use either the above, or, preferably, some one of the anilin colors. Specimens hardened in chromic or osmic acid mixtures take carmine stains badly.

"*c* Clear in either cedar oil, beach wood, creosote or chloroform. Avoid clove oil, as it makes the paraffin granular.

"*d* If the specimen has been stained *in toto*, use Shallibaum's collodion-clove-oil fixative; if sections are to be stained on the slide, use Mayer's albumen fixative.

"*e* If the specimen was stained *in toto*, pass at once to balsam; but if sections are to be stained, pass to absolute alcohol, stain, etc.

"*f* After imbedding and previous to placing in 80 per

cent. alcohol, it is well to place the block for a short time, 1–2 hours, in chloroform. This prevents the formation of bubbles and makes the celloidin more uniform in consistency.

"*g* For routine work use Delafield's hæmatoxylin and eosin-alcohol; this gives a double stain. Any other stain may be used, but some anilins color the celloidin intensely. If other stain than the hæmatoxlyn is used, the eosin-alcohol may be omitted.

"*h* If it is desired to remove the celloidin from section, clean the specimen in clove oil. With delicate section it should not be used."—*Amer. Mon. Mic. Jour. June, 1894.*

56. To remove silver stains from the fingers:

Sulphate of sodium	½ ounce
Chloride of lime	½ ounce
Water	1 ounce

57. To remove pyrogallic avid stains from hands:

Wash with a 10 per cent. solution of oxalic acid, or sulphuric acid diluted with water 1:20.

APERTURE TABLE.

[Copied from Journal of Royal Microscopical Society.]

Numerical Aperture ($n \sin u = a$.)	Correspond'g Angle ($2u$) for Air ($n=1{\cdot}00$).	Correspond'g Angle ($2u$) for Water ($n=1{\cdot}33$).	Correspond'g Angle ($2u$) for Homogeneous Immersion ($n=1{\cdot}52$).	Limit of Resolving Power in Lines to an Inch: White Light ($\lambda=0{\cdot}5269\ \mu$, Line E.)	Limit of Resolving Power in Lines to an Inch: Monochromatic (Blue) Light ($\lambda=0{\cdot}4861\ \mu$, Line F.)	Limit of Resolving Power in Lines to an Inch: Photography ($\lambda=0{\cdot}4000\ \mu$, Near Line h)	Illuminating Power (a^2)	Penetrating Power $\left(\frac{1}{a}\right)$
1·52	.....	.....	180° 0′	146,543	158,845	193,037	2·310	·658
1·51	.....	.....	166° 51′	145,579	157,800	191,767	2·280	·662
1·50	.....	.....	161° 23′	144,615	156,755	190,497	2 250	·667
1·49	.....	.....	157° 12′	143,651	155,710	189,227	2·220	·671
1·48	.....	.....	153° 39′	142,687	154,665	187,957	2·190	·676
1·47	.....	.....	150° 32′	141,723	153,620	186,687	2·161	·680
1·46	.....	.....	147° 42′	140,759	152,575	185,417	2 132	·685
1·45	.....	.....	145° 0′	139,795	151,530	184,147	2·103	·690
1·44	.....	.....	142° 39′	138,830	150,485	182,877	2 074	·694
1·43	.....	.....	140° 22′	137,866	149,440	181,607	2·045	·694
1·42	.....	.....	138° 12′	136,902	148,395	180,337	2 016	·709
1·41	.....	.....	136° 8′	135,938	147,350	179,067	1·988	·709
1·40	.....	.....	134° 10	134,974	146,305	177,797	1·960	·714
1·39	.....	.....	132° 16	134 010	145,260	176,527	1·932	·719
1·38	.....	.....	130° 26	133,046	144,215	175,257	1·904	·725
1·37	.....	.....	128° 40′	132,082	143 170	173,987	1·877	·729
1·36	.....	.....	126° 58′	131,118	142,125	172,717	1·850	·735
1·35	.....	.....	125° 18	130,154	141,080	171,447	1·823	·741
1·34	.....	.....	123° 40	129,189	140,035	170,177	1·796	·746
1·33	.....	180° 0′	122° 6	128,225	138,989	168,907	1·769	·752
1·32	.....	165° 56′	120° 33	127,261	137,944	167,637	1·742	·758
1·30	.....	155° 38′	117° 35	125,333	135,854	165,097	1·690	·769
1·28	.....	148° 42′	114° 44	123,405	133,764	162 557	1·638	·781
1·26	.....	142° 39′	111° 59′	121,477	131,674	160,017	1·588	·794
1·24	.....	137° 36′	109° 20′	119,548	129,584	157,477	1·538	·806
1·22	.....	133° 4′	106° 45′	117,620	127,494	154,937	1·488	·820
1·20	.....	128° 55′	104° 15	115,692	125,404	152,397	1 440	·833
1·18	.....	125° 3′	101° 50	113,764	123,314	149,857	1·392	·847
1·16	.....	121° 26′	99° 29	111,835	121,224	147,317	1·346	·862
1·14	.....	118° 0	97° 11	109,907	119,134	144,777	1·300	·877
1·12	.....	114° 44′	94° 55	107,979	117,044	142.237	1·254	·893
1·10	.....	111° 36′	92° 43′	106,051	114,954	139,698	1·210	·909
1·08	.....	108° 36′	90° 34′	104,123	112,864	137,158	1·166	·926
1·06	.....	105° 42′	88° 27′	102.195	110,774	134,618	1 124	·943
1·04	.....	102° 53	86° 21′	100,266	108,684	132,078	1·082	·962
1·02	.....	100° 10′	84° 18′	98,338	106,593	129,538	1·040	·980
1·00	180° 0′	97° 31′	82° 17′	96,410	101,503	126,998	1 000	1·000
0·98	157° 2′	94° 56	80° 17′	94,482	102,413	124,458	·960	1·020
0·96	147° 29	92° 24	78° 20′	92,554	100,323	121,918	·922	1·042
0·94	140° 6	89° 56	76° 24′	90,625	98,223	119,378	·884	1·064
0·92	133° 51	87° 32′	74° 30′	88,697	96,143	116,838	·846	1·087
0·90	128° 19	85° 10	72° 36′	86,769	94,053	114,298	·810	1·111
0·88	123° 17′	82° 51′	70° 44′	84,841	91,963	111,758	·774	1·136
0·86	118° 38	80° 34	68° 54′	82,913	89.873	109,218	·740	1·163
0·84	114° 17	78° 20′	67° 6′	80,984	87,783	106,678	·706	1·190
0·82	110° 10′	76° 8′	65° 18′	79,056	85,693	104,138	·672	1 220
0·80	106° 16	73° 58′	63° 31′	77.128	83,603	101,598	·640	1·250
0·78	102° 31	71° 49	61° 45′	75,200	81,513	99,058	·608	1·282
0·76	98° 56	69° 42	60° 0	73,272	79,423	96,518	·578	1·316
0·74	95° 28′	67° 37	58° 16′	71,343	77,333	93,979	.548	1·351
0·72	92° 6′	65° 32	56° 32′	69,415	75,242	91,439	·518	1·389
0·70	88° 51′	63° 31′	54° 50′	67,487	73,152	88,899	·490	1·429
0·68	85° 41′	61° 30′	53° 9	65,559	71,062	86,359	·462	1·471
0·66	82° 36′	59° 30′	51° 28′	63,631	68,972	83,819	·436	1·515
0·64	79° 36′	57° 31′	49° 48′	61,702	66,882	81,279	·410	1·562
0·62	76° 38′	55° 34′	48° 9	59,774	64,792	78,739	·384	1·613
0·60	73° 44′	53° 38′	46° 30′	57,846	62,702	76,199	·360	1·667
0·58	70° 54	51° 42′	44° 51′	55,918	60,612	73,659	·336	1·724
0·56	68° 6	49° 48′	43° 14′	53,990	58,522	71,119	·314	1·786
0·54	65° 22	47° 54′	41° 37′	52,061	56,432	68,579	·292	1·852
0·52	62° 40	46° 2′	40° 0′	50,133	54,342	66,039	·270	1·923
0·50	60° 0	44° 10′	38° 24′	48,205	52,252	64,499	·250	2·000
0·45	53° 30	39° 33′	34° 27′	43,385	47,026	57,149	·203	2·222
0·40	47° 9	35° 0′	30° 31′	38,564	41,801	50,799	·160	2·500
0·35	40° 58	30° 30′	26° 38′	33,744	36,576	44,449	·123	2·857
0·30	34° 56	26° 4′	22° 46′	28,923	31,351	38,099	·090	3·333
0·25	28° 58	21° 40′	18° 56′	24,103	26,126	31,749	·063	4·000
0·20	23° 4	17° 18′	15° 7′	19,282	20,901	25,400	·040	5·000
0·15	17° 14	12° 58′	11° 19′	14,462	15,676	19,050	·023	6·667
0·10	11° 29	8° 38′	7° 34′	9,641	10,450	12,700	·010	10·000
0.05	5° 44	4° 18′	3° 46′	4,821	5,252	6,350	·003	20·000

COMPARISON OF ENGLISH AND METRIC MEASURES.

[Behren's Guide to the Microscope in Botany.]

μ	Inches	mm.	Inches	mm.	Inches
1	.000039	1	.039370	51	2.0079
2	.000079	2	.078741	52	2.0473
3	.000118	3	.118111	53	2.0866
4	.000157	4	.157482	54	2.1260
5	.000197	5	.196852	55	2.1654
6	.000236	6	.236223	56	2.2047
7	.000276	7	.275593	57	2.2441
8	.000315	8	.314963	58	2.2835
9	.000354	9	.354334	59	2.3229
10	.000394	10 (1 cm.)	.393704	60 (6 cm.)	2.3622
11	.000433	11	.433075	61	2.4016
12	.000472	12	.472445	62	2.4410
13	.000512	13	.511816	63	2.4803
14	.000551	14	.551186	64	2.5197
15	.000591	15	.590556	65	2.5591
16	.000630	16	.629927	66	2.5985
17	.000669	17	.669297	67	2.6378
18	.000709	18	.708668	68	2.6772
19	.000748	19	.748038	69	2.7166
20	.000787	20 (2 cm.)	.787409	70 (7 cm.)	2.7559
21	.000827	21	.826779	71	2.7953
22	.000866	22	.866150	72	2.8347
23	.000906	23	.905520	73	2.8740
24	.000945	24	.944890	74	2.9134
25	.000984	25	.984261	75	2.9528
26	.001024	26	1.023631	76	2.9922
27	.001063	27	1.063002	77	3.0315
28	.001102	28	1.102372	78	3.0709
29	.001142	29	1.141743	79	3.1103
30	.001181	30 (3 cm.)	1.181113	80 (8 cm.)	3.1496

Inches, &c., into Micro-Millimeters, Etc.	
Inches	μ
$\frac{1}{25000}$	1.0160
$\frac{1}{20000}$	1.2700
$\frac{1}{15000}$	1.6933
$\frac{1}{10000}$	2.5400
$\frac{1}{9000}$	2.8222
$\frac{1}{8000}$	3.1750
$\frac{1}{7000}$	3.6285
$\frac{1}{6000}$	4.2333
$\frac{1}{5000}$	5.0800
$\frac{1}{4000}$	6.3499
$\frac{1}{3000}$	8.4666
$\frac{1}{2000}$	12.6999
$\frac{1}{1000}$	25.3998
	mm.
$\frac{1}{900}$	.0282
$\frac{1}{800}$	.0318
$\frac{1}{700}$	.0363
$\frac{1}{600}$	.0423
$\frac{1}{500}$	.0508
$\frac{1}{450}$	.0564
$\frac{1}{400}$	.0635
$\frac{1}{350}$	.0726
$\frac{1}{300}$	.0847
$\frac{1}{250}$	.1016
$\frac{1}{200}$	.1270
$\frac{1}{150}$	.1695
$\frac{1}{100}$	.2540
$\frac{1}{50}$	.5080
$\frac{1}{25}$	1.0160
$\frac{1}{20}$	1.2700

31	.001220	31	1.220483
32	.001260	32	1.259854
33	.001299	33	1.299224
34	.001339	34	1.338595
35	.001378	35	1.377965
36	.001417	36	1.417336
37	.001457	37	1.456706
38	.001496	38	1.496076
39	.001535	39	1.535447
40	.001575	40 (4 cm.)	1.574817
41	.001614	41	1.614188
42	.001654	42	1.653558
43	.001693	43	1.692929
44	.001732	44	1.732299
45	.001772	45	1.771669
46	.001811	46	1.811040
47	.001850	47	1.850410
48	.001890	48	1.889781
49	.001929	49	1.929151
50	.001969	50 (5 cm.)	1.968522
60	.002362		
70	.002756		
80	.003150		
90	.003543		
100	.003937		
200	.007874		
300	.011811		
400	.015748		
500	.019685		
600	.023622		
700	.027559		
800	.031496		
900	.035433		
1000 (= 1 mm.)			

81	3.1890	$\frac{1}{16}$	1.5875
82	3.2284	$\frac{1}{16}$	1.6933
83	3.2678	$\frac{1}{12}$	2.1166
84	3.3071	$\frac{1}{10}$	2.5400
85	3.3465	$\frac{1}{8}$	3.1750
86	3.3859	$\frac{1}{6}$	4.2333
87	3.4252	$\frac{3}{16}$	4.7625
88	3.4646	$\frac{1}{5}$	5.0800
89	3.5040	$\frac{1}{4}$	6.3499
90 (9 cm.)	3.5433	$\frac{5}{16}$	7.9374
91	3.5827	$\frac{3}{8}$	9.5249
92	3.6221		cm.
93	3.6615	$\frac{7}{16}$	1.1112
94	3.7008	$\frac{1}{2}$	1.2700
95	3.7402	$\frac{9}{16}$	1.4287
96	3.7796	$\frac{5}{8}$	1.5875
97	3.8189	$\frac{11}{16}$	1.7462
98	3.8583	$\frac{3}{4}$	1.9050
99	3.8977	$\frac{13}{16}$	2.0637
100 (10 cm. = 1 decimeter)		$\frac{7}{8}$	2.2225
		$\frac{15}{16}$	2.3812
		1	2.5400
		2	5.0800
		3	7.6200
			decimeter
		4	1.0160
		5	1.2700
		6	1.5240
		7	1.7780
		8	2.0320
		9	2.2860
		10	2.5400
		11	2.7940
		1 foot =	3.0480
		1 yard = 0.9144 meters	

decim.	inches.	
1	3.937043	
2	7.874086	
3	11.811130	
4	15.748173	
5	19.685216	
6	23.622259	
7	27.559302	
8	31.496346	
9	35.433389	
10 (1 meter)	39.370432	
	= 3.280869	feet
	= 1.093623	yards

CHAPTER XIV.

BIBLIOGRAPHY.

The following list of works is given as a guide to the student in the selection of books for the laboratory use. The list is not complete, but it contains some of the most important works for the botanist. Many of these books have been examined by the author for the material he has used in the preparation of this Guide:

Books Relating to Botany.

An Illustrated Encyclopædic Medical Dictionary—*Foster.*

Botany for High Schools and Colleges.—*Bessey.*

Botanical Micro-Technique.—*Zimmermann's, translation by Humphrey.*

College Botany.—*Bastin.*

Comparative Anatomy of Phanerogams and Ferns—*DeBary.*

Contributions from the United States National Herbarium.

Das Mikroskop, Theorie und Auwendung desselben, Nageli und Schwendener.

Elementary Microscopical Technology.—*James.*

Flowers, Fruits and Leaves.—*Lubbuck.*

Guide de l' eleve et du praticien pour les travaux pratiques de micrographie, comprenant la technique et les applications du microscope a l' histologie vegetale, a la physiologie, etc.

Guide to Photo-micrography.—*Bonsfield.*

How to Work with the Microscope.—*Beale.*

Illustrations of North American Wild Grasses.—*Dept. Agri.*

Laboratory Exercises in Botany.—*Bastin.*

Lantern Slides by Photographic Methods.—*Pringle.*

Manual of Botany.—*Gray.*

Manual of Botany, Rocky Mountain Regions.—*Coulter.*

Manual of Plant Histology.—*Dudley.*

Manipulation of the Microscope.—*Bausch.*

Methods of Microscopical Anatomy and Embryology.—*Whitman.*

Microscopist's Vade-Mecum.—*Lee.*

Micro-Chemistry.—*Poulsen, trans. by Trelease.*

Microscopic Dictionary.—*Griffith and Henfrey.*

Nouveau Guide Pratique de Technique Microscophique appliquee a l' Histologie et a l' Embryogenie.—*Boneval.*

Origin of Cultivated Plants.—*DuCaudolle.*

Outlines of Classification and Special Morphology of Plants.—*Goebel.*

Photographic Chemistry.—*Hardwich and Taylor.*

Photographic Instructor.—*Adams and Ehrmann.*

Photo-Micrography.—*Jennings.*

Physiology of Plants.—*Sach.*

Physiology of Plants.—*Vines.*

Physiological Botany.—*Goodale.*

Plant Dissection.—*Arthur, Barnes and Coulter.*

Practical Hints on the Selection and use of the Microscope for Beginners.—*Phin.*

Practical Treatise on the use of the Microscope.—*Queckett.*

Practical Flora.—*Willis.*

Practical Botany.—*Bower and Vines.*

Practical Botany.—*Strasburger.*

Practical Methods in Microscopy.—*Clark.*

Practical Photo-micrography.—*Pringle.*

Practical Guide to Photographic Printing—*Burton.*

Processes of Pure Photography.—*Burton and Pringle.*

Seedlings.—*Lubbuck.*

Sight—An Exposition of the Principles of Monocular and Binocular Vision.—*Joseph LeConte.*

Southern Flora.—*Chapman.*

Structural and Systematic Botany.—*Campbell.*

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Symnoptical Flora of North America.—*Gray.*

Text Book of Botany.—*Vines.*

The Fertilization of Flowers.—*Muller.*

The Leaf-collector's Hand-book.—*Newhall.*

The True Grasses.—*Hackel, trans. by Scribner.*

The Microscope and its Revelations.—*Carpenter.*
The Microscope and Microscope Technology.—*Frey.*
The Microscope and Histological Methods.—*Gage.*
The Microscope.—*Van Heurck.*
The Microscope; its History, Construction and Application. —*Hogg.*
The Student's Manual of Histology.—*Stowell.*
The Art of Projection.—*Dobear.*
The Photographic Negative.—*Burbank.*
The Book of the Lantern.—*Hepworth.*
The Optical Lantern.—*Pringle.*
The Microscope in Theory and Practice.—*Naegeli and Schwendener.*
Treatise on Photography.—*Abney.*
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